***Thermal sensitivity of fertilised eggs is underappreciated cost of extreme heat events in a model insect***

***Electronic Supplementary materials (online version)***

***Materials and methods***

***Set up: thermal sensitivity of eggs to short-duration heat exposure***

Approximately 150 mature adult females were derived from the KSS stock, and paired with a similar number of males (population size: 300 in total within this population to produce adequate numbers of eggs). These beetles were allowed to interact in *ad libitum* fodder medium (~300 grams (g), ~1 gram per beetle) under standard conditions. The beetles were housed in a 1000 ml BPA free food-grade tub (dimensions: 10 x 10 x 12 cm) and females oviposited into this fodder medium. Standardised egg collections were performed after a 72 h adult interaction phase. Adults were separated after this phase by manually sieving the fodder containing unhatched freshly oviposited eggs (at 30°C, within 72 h from adult separation). Oviposited eggs were split equally (using a standard measuring scoops, 15 g & 7.5 g) across multiple replicate batches randomly allocated to different short-duration thermal stress treatments maintained in a two climate chambers (details below). The number of offspring that eclosed successfully (under standard conditions, see main manuscript) after the thermal stress was counted as the measure of fitness and compared to the control offspring eclosion at 30°C ± 1°C.

***Thermal stress exposures***

Short-durations of thermal stress (experienced during heatwaves) were applied using Binder climate chambers (models: KBWF240 and KBWF720, RH 60%; see: https://www.binder-world.com/uk-en/search?tx\_solr%5Bq%5D=KBWF720) to maintain tighter controls on the abiotic conditions (homogeneous temperature conditions are effectively maintained via circulation of warm air by adjustable fans).

***Sampling fertilised eggs post-mating***

Fertilised eggs were assayed (with 7.5 g and 15 g of fodder using a standard measuring scoops) using the same stock population. Eggs were oviposited naturally post-mating were exposed to a 2 h short-duration thermal stress (at 46°C, 48°C and 50°C, ±1°C). In our preliminary assays, we found out that fertilised eggs exposed to 50°C experienced 100% mortality. Subsequent protocols were tuned the thermal stress exposures further to include control conditions: 30°C (±1°C), thermal stress: 46°C (±1°C) and 48°C (±1°C) for 2h and 5h respectively. In a final experiment, egg assays (above) were repeated again using adults from a different generation with half the amount of fodder (7.5 g) to capture the egg responses to the short-term thermal stress.

***Sampling and anonymisation protocol***

Sample sizes for the standardised egg assays (using 15 g fodder, experiment 1) were (N=2 replicate batches for 2 h, trial 1) across 30°C, 48°C and 50°C (experiment 1, trial 1) and 30°C, 46°C and 48°C and [N=5 replicate batches for 2h and 5h, experiment 1, trial 2]. For 7.5 g fodder assay (N=5 replicate batches for 2 h and 5 h; [experiment 2, trial 1]). All the vials were coded, and treatments were anonymised (to all), to minimise any biases through handling and counting. De-anonymisation was undertaken once all the data was collected, prior to statistical analysis.

***Repeatability of egg measures across replicates: (see, main sections for figures)***

From the replicate experiments above, offspring counts (mean ± SE) under control conditions (30°C) under standardised egg collections (in 15 g of fodder, experiment 1) resulted in 222 ± 5.0, [Fig. 2A, trial 1]; 184.6 ± 10.5; 176 ± 7.3 [Fig. 2B, trial 2] offspring. In 7.5 g of fodder (experiment 2), under control conditions at 30°C, reduced amount of fodder resulted in lower offspring numbers as expected [trial 1, mean offspring ± SE = 2 h: 67 ± 6.5 and 5 h: 74 ± 4.5, Fig. 2C].