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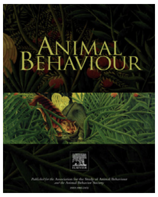

Facultative polyandry protects females from compromised male fertility caused by heatwave conditions

R. Vasudeva*, [1], M. Dickinson [1], A. Sutter, S. Powell, K. Sales, M. J. G. Gage*

School of Biological Sciences, University of East Anglia, Norwich, U.K.

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Why is polyandry such a common mating behaviour when it exposes females to a range of significant fitness costs? Here, we investigated whether polyandry protects females against reduced male fertility caused by thermal stress from heatwave conditions. Sperm production and function are vulnerable to heat, and heatwave conditions are forecast to increase as our climate warms, so we examined these effects on female reproduction and mating behaviour in the flour beetle, *Tribolium castaneum*, a promiscuous ectotherm model in which fertility is damaged by environmental warming. We tested whether polyandrous matings, or polyandrous sperm stores, protect females against reduced male fertility caused by heatwave conditions, and whether females flexibly adjust their remating behaviour to enable fertility rescue. We found that polyandry protected females against reduced male fertility: monogamous matings with males exposed to heatwave conditions halved female offspring production, but opportunities to mate with five of these males allowed normal female reproductive output. By contrast with this fertility improvement following polyandrous mating opportunities, there was no protective benefit for females already carrying sperm stores from multiple males, which suffered similar heatwave damage within the female tract as monogamous sperm stores. Importantly, female polyandry was flexible to male condition, with females showing greater motivation to remate with new males if their previous mate had been damaged by heatwave exposure, enabling a rapid reproductive rescue. Our results reveal that flexible polyandry enables females to rescue their fertility when male reproductive function is compromised by heatwave conditions, a phenomenon that may become more prevalent under climate change.

Polyandry, when females mate with multiple males (Hosken & Stockley, 2003; Pizzari & Wedell, 2013), ‘is both ubiquitous and common in nature’ (Taylor, Price, & Wedell, 2014, page 377). In a comprehensive review exploring 203 population estimates of multiple paternity using microsatellites in 160 animal species, polyandry was found in 89% of populations (Taylor et al., 2014). This ubiquity is puzzling, because polyandry increases the opportunity for sexual conflict by exposing females to a recognized range of significant fitness costs, including vulnerability to predation or abiotic stress, time wasting, reduced paternal care, desertion or infanticide, male manipulation, disease transmission, mate incompatibility, physical trauma and even death (Arnqvist & Rowe, 2005; Parker, 2006). Polyandry also enables sexual antagonism to operate within the genome (Arnqvist & Rowe, 2005; Parker, 2006). Why therefore, given the generally high reproductive potential of individual males, and the widespread costs to females of mating with multiple males, is polyandry such a common mating pattern throughout the animal kingdom? Here, we experimentally investigated whether polyandry can protect females against risks of reduced fertility following thermal stress from heatwave conditions, extreme weather events which are forecast to increase in a warming world (Christidis, Jones, & Stott, 2015; IPCC, 2007; Otto, 2016; Raftery, Zimmer, Frierson, Startz, & Liu, 2017; Yin et al., 2018).

Explanations for the adaptive significance of polyandry can be grouped broadly into three themes: (1) convenience polyandry, (2) indirect benefits and (3) direct benefits. (1) Convenience polyandry occurs when females allow multiple mating as a form of damage limitation against the costs of resisting male sexual harassment (Boulton, Zuk, & Shuker, 2018). Most operational sex ratios mean that males are selected to mate at a higher rate than females, which creates sexual conflict over mating frequency (Bateman, 1948; Trivers, 1972; Kvarnemo & Simmons, 2013). Convenience

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* Corresponding authors.
E-mail addresses: r.vasudeva@uea.ac.uk (R. Vasudeva), m.gage@uea.ac.uk (M. J. G. Gage).

[1] These authors contributed equally.

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polyandry can partly resolve this sexual conflict if the costs to females of accepting multiple matings are lower than the costs of resisting; empirical evidence for this situation exists, for example, in the mating struggles of water striders (e.g. Rowe, Arnqvist, Sih, & Krupa, 1994; Boulton et al., 2018). (2) Polyandry can also provide indirect benefits if multiple mating allows females to harvest 'good' genes for their offspring. Polyandry will strengthen sexual selection, heightening the struggle between males to reproduce, thereby applying a tighter filter on the male breeding population and theoretically allowing females to capture better genes for their offspring. Empirical evidence from a number of systems and scenarios shows that, despite the short-term costs (Arnqvist & Rowe, 2005), polyandry can indeed provide females with longer-term genetic benefits (e.g. Jennions and Petrie, 2007, Radwan, 2004, Neff and Pitcher, 2004, Tregenza & Wedell, 1998, Michalczyk et al., 2011, Price, Hodgson, Lewis, Hurst, & Wedell, 2008, Taylor et al., 2014, Lumley et al., 2015, Godwin, Lumley, Michalczyk, Martin, & Gage, 2020), including under selection from thermal stress (Parrett & Knell, 2018). (3) Finally, polyandry can confer direct benefits to females if multiple mating is associated with increases in male reproductive investment via paternal care, protection or nuptial feeding (Gwynne, 1984; Arnqvist & Nilsson., 2000; Simmons, 2005), or through the assurance of functional sperm and fertility (Wetton & Parkin, 1991; Griffith, 2007; Hasson & Stone, 2009; Pizzari, Løvlie, & Cornwallis, 2004; Radwan, 2003; bib_Reinhardt_and_Ribou_2013Reinhardt & Ribou, 2013; Sheldon, 1994; Sutter et al., 2019). In this study, we examined whether risks of poor male fertility and sperm damage from elevated environmental temperature can be countered by polyandry.

Infertility has been documented across a range of invertebrate and vertebrate taxa in nature (García-González, 2004; Rhainds, 2010), so polyandry could exist as a form of insurance for females against mating exclusively with an infertile or subfertile male (Griffith, 2007; Hasson & Stone, 2009; Sheldon, 1994). Females will clearly suffer profound reproductive costs from lineage extinction if they are paired exclusively with a male that is unable to fertilize, so the evolution of polyandry has been theorized as one mechanism to guard against the risk of female reproductive failure (Hasson & Stone, 2009; Sheldon, 1994; Yasui & Yoshimura, 2018). Comparative analyses across 58 bird species, where egg hatching success can be readily measured, showed that an average of 12% of all eggs failed to hatch in nature (although relationships with mating patterns were not evident; Morrow, Arnqvist, & Pitcher, 2002). In a study across 30 insect species in eight Orders, the median percentage of infertile matings that failed to yield offspring was 22% (García-González, 2004). There is, therefore, evidence for some risk of compromised fertility in nature and, although poor fertility can occur for a number of reasons (Morrow et al., 2002), it is clear that females will be under strong selection to defend their significant reproductive investment against any risk of compromised sperm function (Griffith, 2007; Hasson & Stone, 2009; Sheldon, 1994).

One environmental factor that can compromise male fertility through damage to sperm production and function is thermal stress. It has long been established that male fertility in endotherms is sensitive to the universal abiotic variable of temperature, and studies in mammals and birds have shown that environmentally relevant increases in thermal regime can diminish male reproductive potential (e.g. Thonneau, Bujan, Multigner, & Mieusset, 1998; Setchell, 2006; Paul, Melton, & Saunders, 2008; Hansen, 2009; Hurley, McDiarmid, Friesen, Griffith, & Rowe, 2018). In many mammalian species, for example, the testes are external to the body, allowing them to be maintained at 2–8 °C below core temperature (Harrison & Weiner, 1948; Banks, King, Irvine, & Saunders, 2005), and experiments have shown that this testicular cooling is essential to maintain normal fertility in such species (Mieusset et al., 2006; Skinner & Louw, 1966; Wettemann, Wells, Omtvedt, Pope, & Turman, 1976; Jannes et al., 1998; Banks et al., 2005). A number of studies have shown that environmentally relevant warming can damage male fertility (Burfening, Elliott, Eisen, & Ulberg, 1970; Tablado, Revilla, & Palomares, 2009; López-Gatius, 2003), and this male reproductive sensitivity to heat is now increasingly recognized in 'cold-blooded' ectotherms, where physiological function will be more directly influenced by the thermal environment. In *Drosophila melanogaster* fruit flies, for example, population viability drops off above 30 °C, because this is the rearing temperature at which males typically become sterile (David et al., 2005), and male thermal fertility limits can predict drosophilid species distribution better than viability limits (Parratt et al., 2020).

Likewise, in *Tribolium* flour beetles, experimental heatwave conditions that are 5–7 °C above the population productivity optimum of 35 °C damage sperm production and viability, causing reduced fertility and reductions in offspring fitness (Sales et al., 2018, 2021). A single such heatwave halves fertility, while a second heatwave renders males almost sterile, whereas females are resilient to heat stress unless they are carrying sperm from previous matings, which become damaged if they suffer heat exposure in storage (Sales et al., 2018, 2021). Because fertility can be compromised by the thermal environment, and because climate change will create a greater incidence of more extreme heatwave conditions, we therefore explored whether polyandry can shield females from male reproductive damage caused by heatwave conditions. Using the model insect *Tribolium castaneum*, representative of a huge number of coleopteran species, we examined whether (1) polyandrous mating opportunities or (2) storing sperm from multiple males can protect females from reduced male fertility or sperm damage caused by heatwave exposure. Having established how polyandry benefits females under heatwave-induced compromised fertility, we then tested the hypothesis that females show facultative polyandry, flexibly modifying their remating behaviour depending on the heatwave exposure and fertility status of their previous mate. Our study closely follows that by Sutter et al. (2019) who examined flexible polyandry in relation to heat stress and male infertility in *Drosophila pseudoobscura*. However, as well as confirming the general findings of Sutter et al. (2019) in a different model system, we also assessed the 'postcopulatory' benefits of polyandrous sperm stores for female fertility when sperm are damaged within females by heatwave conditions, and we also examined in more detail how female remating behaviour responds to male infertility, and the specific consequences of that for reproductive rescue. As climate change increases the frequency, duration and intensity of heatwaves (Christidis et al., 2015; IPCC, 2007; Otto, 2016; Raftery et al., 2017; Yin et al., 2018), understanding how reproductive behaviour responds to thermal stress is becoming more relevant (Walsh et al., 2019).

METHODS

Ethical Note

Ethical approval for the research was granted by UEA's Animal Welfare and Ethical Review Board; no invasive procedures were applied, and beetles were maintained and treated under conditions that would be encountered in their natural environment as a human commensal and pest of stored products.

Insect Maintenance

We maintained *T. castaneum* flour beetles from the Krakow Super-Strain (KSS) in a controlled environment at 30 °C and 60 ± 10%

relative humidity under a 16:8 h light:dark photoperiod, as previously described (Dickinson, 2018; Sales et al., 2018; Vasudeva et al., 2019). Small stock populations were reproduced by ca. 600 adults across nonoverlapping generations (ca. 35 days) and maintained with ad libitum fodder consisting of 90% organic flour and 10% brewers' yeast (Doves Farm Foods Ltd, Hungerford, U.K. and ACROS Organics, Antwerp, Belgium) topped with a layer of oats for traction. We obtained experimental beetles by isolating and sexing pupae from the stocks and keeping them in single-sex groups of 20 individuals in 6 cm petri dishes with 15 ml of fodder. After adult eclosion, individuals were allowed a further 12 ± 2 days in their single-sex groups to reach reproductive maturity before being randomly assigned to their treatment. During maturation, we marked adults of one sex on the dorsal thorax using a nontoxic water-based marker (Uniposca, www.posca.com) for identification. Experimental treatment groups were blinded or anonymously coded before the start of the assays until the completion of all data collection.

Heatwave Exposure

We exposed sexually mature beetles (12 ± 2 days post adult eclosion) in single-sex groups to heatwave conditions for 5 days at 42 ± 1 °C and 60 ± 10% relative humidity, 7 °C above the 35 °C optimum for population productivity in *T. castaneum* (Mickel & Standish, 1947, Howe, 1956; Sokoloff, 1974). This thermal treatment consistently damages the subsequent fertility of adult males (Sales et al., 2018, 2021). Heatwaves are commonly defined when temperatures exceed the normal average maxima by 5 °C or more for at least 5 days (Frich et al., 2002), although variability will obviously occur in the natural environment compared with our stable experimental conditions. Temperature extremes of 42 °C or more have been recorded in the natural environment across 103 countries (Mherrera, 2016). Heatwave conditions were applied using an A.B. Newlife 75 Mk4 forced-air incubator (A.B. Incubators, Ipswich, U.K.). Control beetles were treated the same as heat-exposed beetles, except kept throughout at 30 °C. Any males that died were discarded, with the heatwave treatments resulting in the deaths of 29 of 300 males (9.6%) compared to 21 of 240 controls (8.8%); there was no significant difference in survival between the two treatments (median survival for both treatments 90%; quasibinomial generalized linear model, GLM: b [~95% confidence interval, CI] = −0.11 [−0. 83, 0.61], $t = -0.31$, $P = 0.763$; Fig. A1). Treatment temperatures were within 1 °C of the experimental set point, checked using a 35–45 °C mercury incubation thermometer (G.H. Zeal Ltd, London, U.K.) calibrated to United Kingdom Accredited Service standards (Charnwood Instrumentation Services Ltd, Coalville, U.K.). Following heat stress and control treatments, we maintained all individuals in common garden conditions at 30 ± 1 °C for 24 h, before being used in breeding experiments at 30 ± 1 °C.

Benefits of Polyandry When Males Experience Heatwaves

We gave reproductively mature adult KSS females that had been maintained throughout in standard 30 °C conditions mating opportunities with either a single mature male (monandrous regime) or five mature males (polyandrous regime). All adults were unmated and 18 ± 2 days posteclosion at the start of the reproductive fitness assay, and were given a 48 h mating opportunity in either 7 ml vials with 3 ml of fodder (monandrous pairs) or 5 cm petri dishes and 15 ml of fodder (polyandrous groups). As *T. castaneum* displays promiscuous mating behaviour, exposure of females to five males will typically result in sperm storage from multiple males (Pai & Yan, 2003; Fedina & Lewis, 2008; Lumley et al., 2015). Males exposed to heatwave conditions also readily mate and inseminate females across a 48 h mating opportunity, even though their sperm may be compromised (Sales et al., 2018). In approximately half of these monandrous and polyandrous treatments, the males had been exposed to 5 days of 42 °C heatwave conditions which had completed 24 h before the start of mating opportunities, and the other half were age-matched control males that had been maintained throughout at 30 °C. Following the 48 h monandrous or polyandrous mating opportunity, we moved females to oviposit individually in 5 cm petri dishes containing 15 ml of fodder for 20 days, in two 10-day oviposition blocks to prevent overlapping generations and limit larval competition, before discarding them. We therefore scored reproductive output of each female as the number of offspring successfully produced over 20 days of oviposition (Sales et al., 2018), which represents half of a female's lifetime offspring production under these conditions, and also correlates significantly with her total offspring production under these conditions ($R^2 = 0.55$, $P = 0.001$, $N = 46$, Figure 2.3 in Dickinson, 2018). We compared reproductive fitness between females that had been given mating access to either 30 °C control or 42 °C thermal treatment males, in either monandrous or polyandrous conditions (Fig. 1a ; monandrous mating opportunities with a 30 °C control male: $N = 57$; monandrous mating opportunities with a 42 °C heat stress male: $N = 59$; polyandrous mating opportunities with 30 °C control males: $N = 56$; polyandrous mating opportunities with 42 °C heat stress males: $N = 60$).

Benefits of Polyandry When Stored Sperm Experience Heatwaves

As well as disruption of sperm development and viability in males, the function of sperm stored within the female reproductive tract after insemination is compromised by heat stress exposure of females, halving future offspring production (Sales et al., 2018, 2021). We therefore measured whether sperm stores in females given the opportunity to mate polyandrously were more resilient to fertility damage from thermal stress, compared with those of females storing sperm following monandrous mating opportunities with single males. As above, females were provided with 48 h mating opportunities with control males that had been maintained throughout at 30 °C, either with single males (to generate monandrous sperm stores) or five males (to generate polyandrous sperm stores). Assays of female mating behaviour show that females will mate with multiple males under these conditions (Michalczyk et al., 2011), and that males can inseminate multiple females (Lumley et al., 2015). After the 48 h mating opportunities, we discarded the males. Approximately half of the females were exposed to 5-day 42 °C heatwave conditions, and the other half to standard 30 °C control conditions, after which all were maintained for 24 h in a common garden environment of 30 °C. Following this treatment, females were moved to oviposit individually in 5 cm petri dishes containing 15 ml of fodder for two 10-day periods, with the resulting reproductive output scored as the number of adult offspring emerging. Reproductive output of each female was therefore the number of offspring successfully produced over 20 days of oviposition (Sales et al., 2018), and we compared the fitness of females that were either carrying monandrous or polyandrous sperm stores, following 30 °C control or 42 °C heatwave conditions (Fig. 1b; monandrous sperm stores exposed to 30 °C conditions: $N = 46$; monandrous sperm stores exposed to 42 °C heat stress: $N = 32$; polyandrous sperm stores exposed to 30 °C conditions: $N = 43$; polyandrous sperm stores exposed to heat stress: $N = 40$).

Female Remating Behaviour

To assess whether females use flexible polyandry to improve their reproductive output after mating with a male whose fertility had (potentially) been compromised by heat exposure,

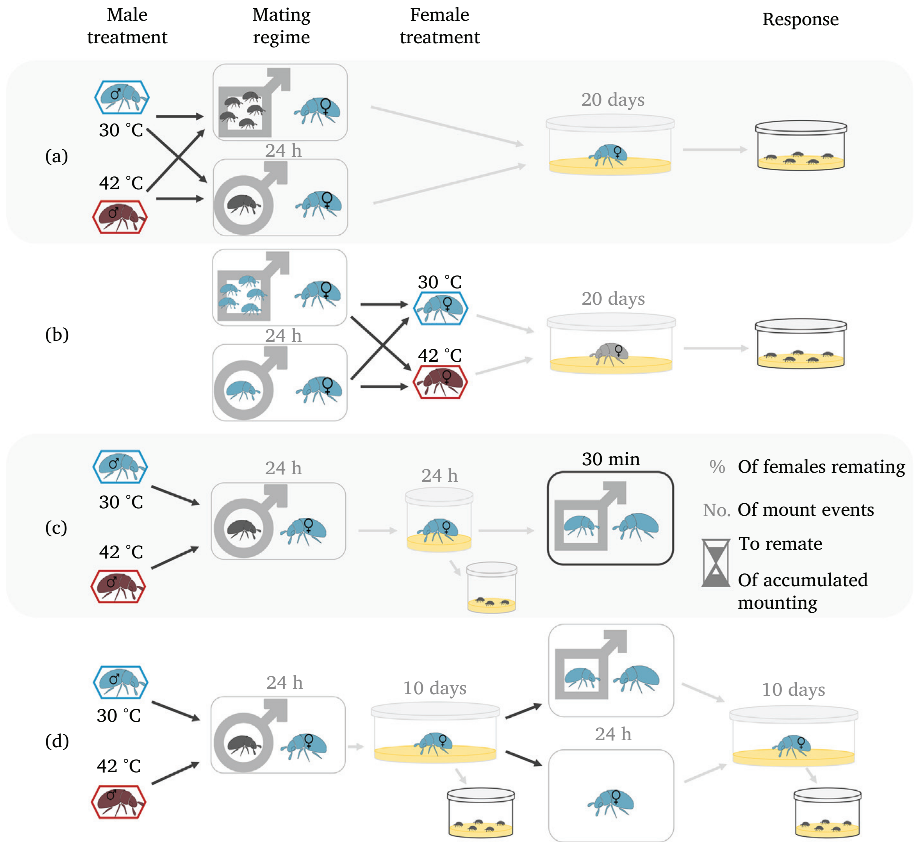

Figure 1. Design of four experiments investigating the adaptive significance of polyandry in response to damaged male fertility due to heatwaves (42 °C). Central factors (mating regime, temperature treatment) of the fully factorial design as well as reponse variables of interest (reproductive output, remating behaviour) are highlighted in dark grey. Polyandrous mating opportunities are represented with square male symbols (consistent with Figs. 2–5). (a) Female reproductive fitness outcome following mating opportunities with control males (blue, 30 °C) or heatwave-exposed males (dark red, 42 °C) under polyandry or monogamy. (b) Reproductive fitness outcome of females exposed to heatwaves with polyandrous or monogamous sperm stores. (c) Behavioural observations of females after being mated with a control or heatwave-exposed male. (d) Fitness consequences of flexible polyandrous remating among females that were initially mated to a control or a heatwave-exposed male.

we conducted female remating behaviour and reproductive fitness rescue trials. The remating behaviour trials were conducted in three separate experimental blocks, with each block using beetles bred from a different generation and stock. To minimize observer bias, we coded experimental females prior to the behavioural assays so their treatment group could not be identified during the behavioural assays. We paired unmated adult females (18 ± 2 days posteclosion) monandrously for 24 h mating opportunities in 7 ml vials containing 3 ml of fodder with either (1) a male that had been exposed to 5 days of 42 °C heat stress conditions (completed 24 h before the start of the assay) or (2) a control male that had been maintained at 30 °C throughout. Males were all unmated and 18 ± 2 days posteclosion. We separated pairs after 24 h to allow the females to oviposit in isolation, and a further 24 h later all females were provided with a second tester male to record remating behaviour. The tester males were all unmated, 18 ± 2 days posteclosion, and maintained throughout at 30 °C. Following introduction of both adults to an empty 7 ml vial, we observed each pair for 30 min at 30 °C and 60% relative humidity (in batches of 5–10 experimental pairs per observer). Mating success, latency to mate, the number of copulatory mounts and copulation duration were recorded for each pair (Fig. 1c). We scored successful matings when copulatory coupling lasted more than 33 s, which is the average minimum mating period indicating successful spermatophore transfer in *T. castaneum* (Edvardsson and Arnqvist, 2005; Droge-Young, Belote, Perez, & Pitnick, 2016).

To establish whether previous complete mating failure could influence our remating behaviour results, we incubated the vials that our tester females oviposited in between their first and second mating opportunity, and counted emerged offspring after 35 days of incubation. In addition, we directly assayed the frequency of sperm transfer for a random subset of 24 females paired monogamously with males that had been previously exposed to 42 °C heat stress conditions. After the females had had a 24 h mating opportunity at 30 °C, we decapitated them and placed them on a clean glass slide for dissection in a 30 μl drop of insect saline (0.9% NaCl) under an Olympus SZX9 binocular microscope. The entire reproductive tract was isolated and removed, placed under a 22 × 22 mm cover slip and scored for the presence of spermatophores in the bursa and/or sperm in the bursa or spermatheca (Sales et al., 2018).

Reproductive Fitness Rescue Following Remating

To measure the fitness benefits of female remating, we ran two additional experimental blocks in which we first gave females 24 h mating opportunities with either a single control or 42 °C heatwave-exposed male, as described above. To measure reproductive fitness consequences of remating opportunities, we provided these females with a 30 min remating opportunity with a new control male (behaviour not recorded), after they had been ovipositing alone for 10 days (in a 5 cm petri dish with 15 ml of fodder). After the 30 min remating opportunity, females were returned to a new labelled 5 cm petri dish with 15 ml of fodder for another 10-day oviposition period under standard conditions. Offspring produced from this oviposition period were counted when they emerged (35 days later) as adults, and we used this score to compare reproductive fitness benefits of remating for females after they had been initially paired with either heatwave-exposed or control males (Fig. 1d). To compare fitness benefits of remating versus no remating, a control set of females were exposed in parallel to the same initial mating opportunities (males exposed to 42 °C heatwaves versus 30 °C controls), but then denied the opportunity to remate, providing baseline reproductive output data under enforced monogamy following pairing with heatwave-exposed versus normal males.

Statistical Analyses

We carried out all analyses using R Studio (RStudio Team, 2019; v 1.2.5019 and 1.2.5033) in R version 3.6.1 (R Development Core Team, 2017) and figures were generated using the 'ggplot2' (Wickham, 2016) and 'beeswarm' packages (Eklund, 2016). Data sets were summarized using 'tidyverse' (Wickham et al., 2019) and 'Rmisc' (Hope, 2013) packages. Specific approaches for individual results are described below. Across experimental blocks, any replicates where the individual died during an experiment were censored ($N = 18$). Assumptions for data distributions were checked to determine the relationship between the mean and variance of the response variable and the appropriate error distributions (Crawley, 2012), and model residuals were checked for violations of assumptions using the 'DHARMa' package (Hartig, 2020). All biological replication produced here was included in the formal analysis. Where relevant, experimental blocks were added in as a fixed effect with three levels (Thomas et al., 2015).

Mating with heatwave-exposed males

We used a linear model (LM) to analyse female reproductive fitness (total offspring production over two 10-day periods) following monandrous versus polyandrous mating opportunities with heatwave-exposed versus control males. We entered mating regime (monogamous versus polyandrous mating opportunity) and male treatment temperature (30 °C or 42 °C) as predictors, including their interaction.

Heatwave exposure of stored sperm

Similarly, we analysed reproductive fitness of females exposed to heatwave versus control conditions (female temperature) and carrying monandrous versus polyandrous sperm stores (mating regime) with an LM, including female temperature, their mating regime and the interaction between the two as explanatory variables.

Female remating behaviour

The proportion of females that successfully remated was analysed using a quasibinomial GLM ('logit' link) to account for overdispersion, combining females that remated and that did not remate using the cbind function, and including male treatment and experimental block as explanatory variables. Mating latency, the number of mounts and copulation duration of females that mated (221 of 309 females) were analysed with linear mixed-effects models (LMMs) in the 'lme4' package, version 1.1–21 (Bates, Mächler, Bolker, & Walker, 2015). To satisfy assumptions about the distribution of residuals, we log transformed mating latency and copulation duration, and square-root transformed the number of mounts. We included the treatment (control or heatwave) of the female's previous mate and experimental block as fixed effects.

Consequences of female remating

We attempted to analyse female reproductive output after being paired previously with a control or heatwave-treated male using zero-inflated models in 'glmmTMB' (Brooks et al., 2017) with a Gaussian distribution for the conditional part of the model. However, complete separation (see the Hauck–Donner effect) occurred because none of the control females failed to produce offspring after their first mating. Hence, we analysed producing any offspring versus failure to do so as a binary outcome using chi-square tests, and fecundity among females with nonzero offspring counts using LMMs. The full LMM included male treatment (heatwave versus control), mating regime (polyandry versus monandry), egg-laying period (after the second versus first mating opportunity), as well as all possible interactions as fixed effects. We included female ID nested within experimental block as random intercepts. We were not able to fit random slopes between the first and second egg-laying period for individual females due to model convergence issues. We performed backwards model selection to remove nonsignificant terms, starting with interaction terms. We used Satterthwaite's method, implemented in lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017).

RESULTS

Benefits of Polyandry when Males Experience Heatwaves

Polyandrous mating opportunities with standard control males did not change the reproductive output of females compared with those mating monandrously (b [~95% CI] = −1.65 [−27.6, 24.3], $t_{1,228} = -0.125$, $P = 0.901$). However, there was a clear benefit of polyandry for females when breeding with males whose reproductive potential had been damaged through exposure to 42 °C heatwave conditions, evidenced by a strong interaction between male treatment and mating regime (Fig. 2; $b = 119.0$ [82.8, 155.2],

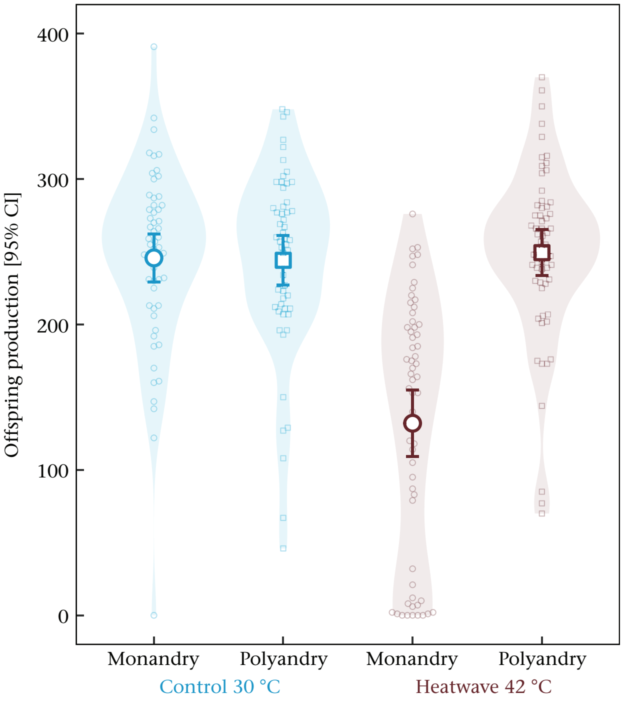

Figure 2. Offspring production of females mated either monandrously (circles) or polyandrously (squares) with males previously exposed to control (blue) or heatwave (dark red) conditions. Large symbols and error bars represent mean and associated approximate 95% confidence intervals, with raw data shown as small symbols.

$t_{1,228} = 6.47$, $P < 0.001$). Polyandrous mating opportunities cancelled out the detrimental effect of male heatwave treatment on monandrous females ($b = 113.7$ [139.3, 88.1], $t_{1,228} = -8.75$, $P < 0.001$), enabling full reproductive rescue. Monandrous matings with heatwave-exposed males resulted in a halving of female reproductive fitness, while opportunities for polyandry enabled females to rescue their reproductive fitness back up to the same levels as those following matings with control males (Fig. 2).

No Benefits of Polyandry for Sperm Stored through Heatwaves

By contrast with our finding that a polyandrous mating opportunity enabled females to rescue their reproductive fitness when male fertility had been compromised by heatwave exposure, we found no evidence for any equivalent protective benefit from holding sperm stores from multiple males (Fig. 3). Previous *T. castaneum* research has shown that heatwave conditions damage sperm in female storage, which then reduces female fertility (Sales et al., 2018). Our results here confirmed that sperm were damaged in storage when females were exposed to heat stress conditions, causing a subsequent halving in female fertility (main effect of temperature: $b = -119.5$ [-143.0, -95.9], $t_{1,157} = -9.96$, $P < 0.001$). Importantly, this fertility reduction occurred irrespective of whether females had been given previous opportunities to store sperm from one versus five males (mating regime: $b = 9.0$ [-12.7, 30.7], $t_{1,157} = 0.82$, $P = 0.417$; interaction: $b = -9.5$ [-42.0, 23.0], $t_{1,157} = -0.57$, $P = 0.568$).

Female Remating Behaviour

Females showed flexible remating behaviour depending on the heatwave exposure and fertility status of their previous mate (Fig. 4). Compared to control females, females that had been given a mating opportunity with a 42 °C heatwave-exposed male (Fig. 2; Sales et al., 2018, 2021) were more likely to remate when provided with a new male (Fig. 4a; quasibinomial GLM: $b = 0.89$ [0.63, 1.15], $t = 6.87$, $P = 0.021$). These females previously paired with a heatwave-exposed male were also quicker to copulate (Fig. 4b; LM on log-transformed latency: $b = -3.10$, [-0.92, -5.29], $t_{1,217} = -2.84$, $P < 0.001$), and engaged in more sexual behaviour with the new male (Fig. 4c; LM on square root-transformed number of mounts: $b = 0.58$, [0.37, 0.79], $t_{1,217} = 5.49$, $P < 0.001$). Once engaged in copula with the new male, mating duration was similar irrespective of whether the female's previous mate had been exposed to a heatwave or not (Fig. 4d; LM on log-transformed duration: $b = 0.05$, [-0.08, 0.18], $t_{1,217} = 0.76$, $P = 0.446$).

Incubating vials that females oviposited in for the first 24 h prior to their second mating opportunity revealed that 73% (127/174) of females produced some viable offspring after being paired with a heatwave-exposed male. Although, this proportion was higher for females paired with control males (126/134 = 94%; $\chi^2_1 = 21.4$, $P < 0.001$), it demonstrates that females did not simply refuse to mate with heatwave-exposed males. Moreover, among females initially paired with heatwave-exposed males, polyandry levels were not significantly higher if females had failed to produce any viable offspring (35/47 = 75% versus 102/128 = 82%; $\chi^2_1 = 0.81$, $P = 0.368$). Finally, our subset of 24 females that were dissected after a 24 h mating opportunity with a heatwave-exposed male showed that 22 (92%) had a spermatophore present in their bursa copulatrix (Fig. A2), similar to levels previously reported (Sales et al., 2018). Thus, a high proportion of females had been mated and inseminated by heatwave-exposed males, and increased polyandry was not simply a result of a higher incidence of previous mating failure.

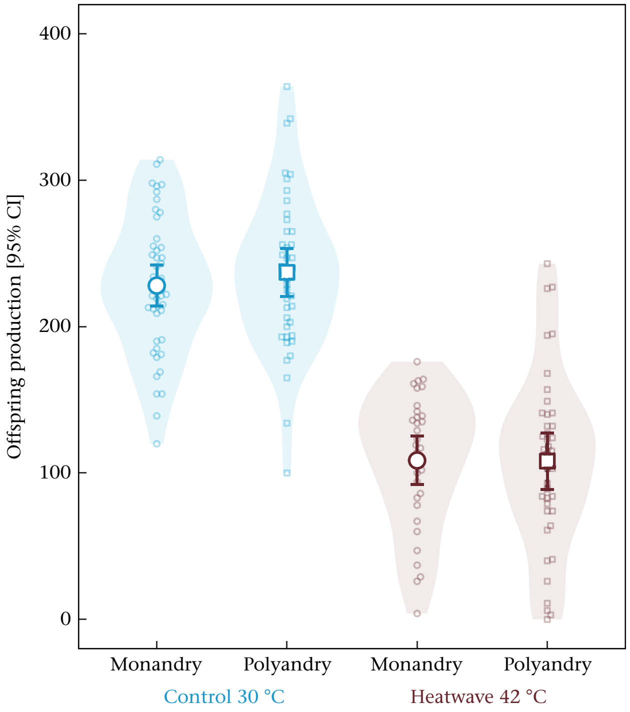

Figure 3. Offspring production of females mated either monogamously (circles) or polyandrously (squares) before being exposed to 30 °C (control, blue) or 42 °C (heatwave, dark red) conditions, respectively. Large symbols and error bars represent mean and associated approximate 95% confidence intervals, with raw data shown as small symbols.

Fitness Consequences of Polyandry

Importantly, those females that had previously been given mating opportunities with a 42 °C heatwave-exposed male gained an immediate reproductive fitness improvement if they were given an opportunity to remate polyandrously with a new control male, restoring offspring production to the same level as that shown after matings with control males with normal fertility (Fig. 5). Only females that had initially been paired with a heatwave-exposed male, but were given an opportunity for polyandry, differed in their likelihhod of having zero offspring between the first and the second 10-day period (25/42 = 60% versus 0/42 = 0%; $\chi^2_1 = 32.8$, $P < 0.001$). Females paired with control males almost never failed to produce offspring, and many females paired with heatwave-exposed males but denied a remating opportunity remained sterile throughout (chi-square tests: all $P > 0.474$). Variation in nonzero fecundity among females was also explained by interactions between male treatment, mating regime and laying period (Fig. A3). Specifically, both the interactions between male treatment and laying period and between mating regime and laying period were retained during model selection ($F_{1,114.3} = 6.55$, $P = 0.012$ and $F_{1,113.5} = 10.1$, $P = 0.002$, respectively). This means that remating with new males was beneficial to females initially mated to heatwave-exposed males because it prevented females from failing to produce any offspring, and additionally increased the fecundity among offspring-producing females (Fig. 5). A similar pattern was seen when females that produced no offspring were excluded (Fig. A3).

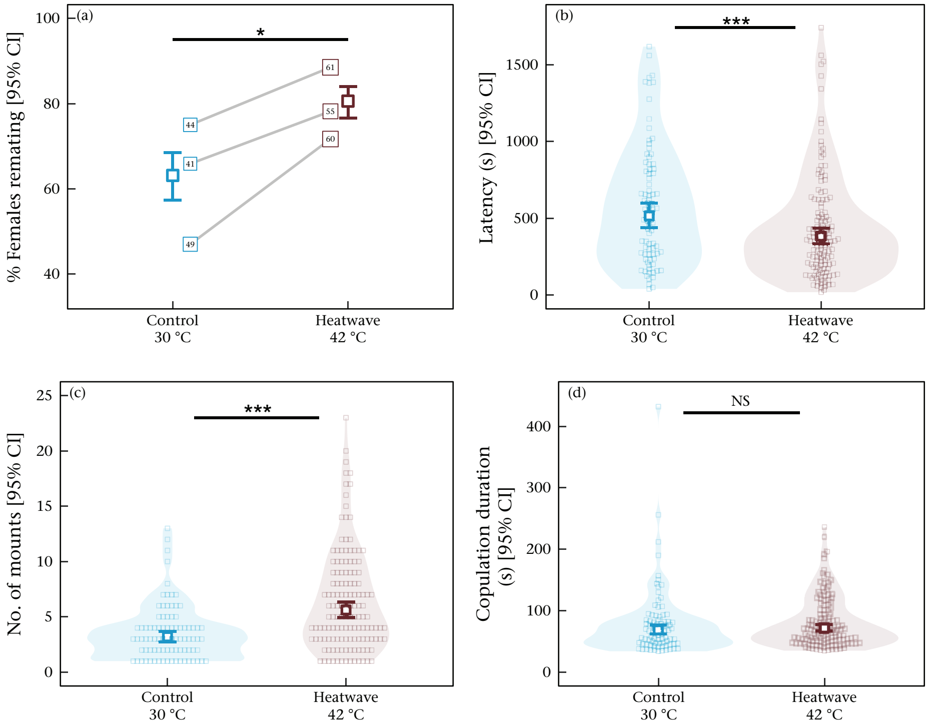

Figure 4. Remating behaviour of females previously mated to males exposed to control (blue) or experimental heatwave conditions (dark red). (a) Percentage of females remating (three experimental replicates connected by grey lines), (b) latency to remate, (c) number of mounts and (d) copulation duration. Larger squares and error bars denote mean and approximate 95% confidence intervals based on (a) model predictions, (b, c) raw data back-transformed from a square-root transformation and (d) raw data back-transformed from a log transformation. (a) Raw data are shown as smaller symbols, with symbol area proportional to sample size (numbers indicated inside squares). (b, c, d) Shaded areas (violin plots) represent the density distribution of raw data. Asterisks indicate significant differences based on (a) generalized and (b, c, d) linear mixed models. *$P < 0.05$; ***$P < 0.001$.

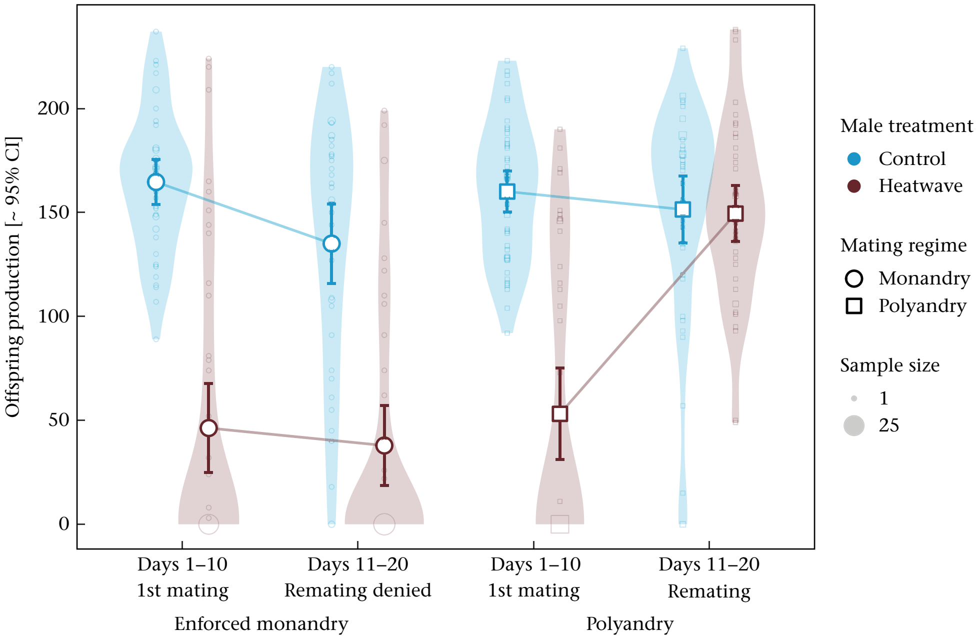

Figure 5. Offspring production of females first mated to control males or heatwave-exposed males and then after 10 days either not allowed to remate (enforced monandry) or allowed to remate (polyandry). Large symbols and error bars show means and approximate 95% confidence intervals. Raw data are shown alongside, with symbol area representing the number of identical outcomes. Figure A3 also presents these data for all females that produced some offspring.

DISCUSSION

Our experiments confirm that reproduction is clearly compromised by male or spermatozoal exposure to experimental heatwave conditions (Sales et al., 2018, 2021), but that opportunities for mating polyandrously can shield females from this reduced male fertility. In the absence of thermal stress, monandrous versus polyandrous mating opportunities provided females with similar levels of full reproductive output (Fig. 2). However, if males' fertility had been compromised as a result of heatwave exposure, mating exclusively with just one male reduced a female's reproductive fitness by a half, whereas females provided with polyandrous mating opportunities with five such males were able to rescue their fertility to normal, full levels (Fig. 2). Importantly, we showed that females gained these fertility benefits by facultatively increasing their rate of polyandry: females remated faster, more often and at a higher rate with a new male if previously paired with males whose fertility had been damaged by heatwave conditions (Fig. 4).

We assessed both ‘copulatory’ and ‘postcopulatory’ reproductive benefits to females from polyandry, and were able to show that it is the opportunity for polyandrous mating, rather than the maintenance of polyandrous sperm stores, that enables these fertility improvements for females. Heatwave exposure to females that had already mated and stored sperm led to a subsequent halving in female reproductive output, irrespective of whether a female's sperm stores had been previously acquired through monandrous or polyandrous mating opportunities (Fig. 3). However, the opportunity to mate polyandrously after males had been exposed to fertility-damaging heatwave conditions allowed females to avoid compromised reproduction (Fig. 2). Heat stress exposure to male *T. castaneum* reduces sperm number, viability and movement through the female reproductive tract (Sales et al., 2018). Polyandrous mating could enable females to either accumulate adequate sperm numbers in the bursa and spermatheca for full fertility or filter damaged or nonviable sperm away from the fertilization set. Once in female storage, however, and irrespective of whether they came from single or multiple males, we found that sperm (and therefore female fertility) were vulnerable to heatwave damage.

Despite the recognized costs (Hosken & Stockley, 2003; Arnqvist & Rowe, 2005; Parker, 2006; Pizzari & Wedell, 2013), widespread polyandry (Taylor et al., 2014) has been theorized to evolve for insurance against risks of male infertility and the termination of a female's reproductive lineage (Sheldon, 1994; Hasson & Stone, 2009; Yasui & Yoshimura, 2018). Natural infertility is widely recognized (García-González, 2004; Rhainds, 2010), and mating with multiple males has been shown to guard against risks of mating failure or male infertility in a range of vertebrate and invertebrate taxa (e.g. Sakaluk & Cade, 1980; Gibson & Jewell, 1982; Wetton & Parkin, 1991; Sheldon, 1994; Delisle & Hardy, 1997; Krokene et al., 1998; Arnqvist & Nilsson, 2000; Pizzari et al., 2004; Uller & Olsson, 2005; Hasson & Stone, 2009; Forbes, 2014), including in *T. castaneum* (Pai, Bennett, & Yan, 2005). Here, we detail the benefits of polyandry for females facing a halving in their reproductive output due to compromised male fertility following thermal stress. Recent work with *D. pseudoobscura* has found similar results (Sutter et al., 2019), with heat-exposed, subfertile males being slower and less successful to mate, and females then showing a doubling in their likelihood of subsequent mating to enable reproductive rescue after first mating with these subfertile males. Research is now needed to measure the prevalence of male fertility damage by thermal extremes in the natural environment, and therefore the extent of natural and sexual selection for polyandry to improve reproductive success following heatwaves.

In addition to showing that polyandry guards females against heatwave-induced poor male fertility, we also found that female *T. castaneum* facultatively vary their remating behaviour with new males depending on their immediate need for fertility rescue. Females previously paired with a male that had experienced a 5-day 42 °C experimental heatwave, and therefore suffering sperm damage and an average halving of reproductive potential (Fig. 2; Sales et al., 2018; Sales et al., 2021), were (1) more likely to remate with a new male, (2) did so sooner when the opportunity was provided and (3) engaged in more sexual behaviour (Fig. 4). Of the females previously mated to a heatwave-exposed male, 80% remated polyandrously within 30 min of observation when given the opportunity of mating with a new male, compared with 63% of females previously paired with a normal fertility male (Fig. 4a). Within these higher remating frequencies, females previously mated to a subfertile, heatwave-exposed male also showed a shorter latency to remate, on average gaining copula within 9 min of the second male's introduction versus almost 13 min for females previously paired to a control male (Fig. 4b). Females that had previously been paired with heatwave-exposed males also displayed greater sexual activity when given access to a second male, engaging in almost twice as many copulatory mounts compared to females previously mated to a normal fertility male (Fig. 4c). Although we can link female remating behaviour to reduced sperm number, viability and presence in the *T. castaneum* female reproductive tract (Sales et al., 2018), there is also the possibility that thermal stress denatures male seminal fluid proteins, which influence female remating behaviour in other insects (e.g. Chapman, 2001; Liu & Kubli, 2003). Notably, we can be sure that these changes in female remating behaviour were not due to mating or insemination failure by their previous heatwave-exposed mates, because dissections showed that more than 90% of these males had successfully transferred spermatophores to females, and most females had some, albeit reduced, reproductive output after mating with a heatwave-exposed male.

To support these findings for facultative polyandry, we also showed that remating by females, even when only given a 30 min opportunity, enabled rapid fertility rescue to normal levels for those females suffering reproductive limitations due to their previous mate's poor fertility. Moreover, for these females previously paired to males that had been exposed to heatwaves, female reproductive output was restored to normal levels for at least 10 days across the second half of a 20-day experimental oviposition period (Fig. 5). These combined findings reveal that females vary their remating behaviour adaptively according to their fertility status, using flexible polyandry to recover reproductive output when it is most needed. Facultative polyandry to enable female fertility rescue or protection has been recorded across a number of conditions and species (e.g. birds: Wetton & Parkin, 1991; reptiles: Friesen, Uhrig, & Mason, 2014; insects: Kraaijeveld & Chapman, 2004, Landeta-Escamilla, Hernández, Arredondo, Díaz-Fleischer, & Pérez-Staples, 2016, Sutter et al., 2019). However, this behaviour may be context- or species-dependent, because other studies have found that female remating behaviour and polyandry are unaffected by fertility or sperm storage status (e.g. Morrow et al., 2002; Harmer, Radhakrishnan, & Taylor, 2006; Abraham et al., 2014; Krüger et al., 2019). Studies of natural systems have even shown that females may preferentially mate with males that confer low fertility, owing to their high-ranking reproductive status causing sperm depletion (Preston, Stevenson, Pemberton, & Wilson, 2001; Warner, Shapiro, Marcanato, & Petersen, 1995).

Conclusions

In summary, we found that female *T. castaneum* used polyandry as a behavioural strategy to improve their reproductive fitness when faced with risks of poor fertility as a consequence of heatwave damage to males and their sperm (Figs. 2 and 3; Sales et al., 2018; Sales et al., 2021). Females deployed this polyandrous behaviour flexibly according to proximate fertility needs (Fig. 4), allowing them to balance the costs of multiple mating (Arnqvist & Rowe, 2005) against the benefits of replenishing reduced, damaged or nonviable sperm stores (Fig. 5). As our climate warms, heightened atmospheric volatility is forecast to increase the frequency, intensity and duration of heatwaves (Otto, 2016, Yin et al., 2018, Christidis et al., 2015, IPCC, 2007, Raftery et al., 2017). Reproductive sensitivity and potential to adapt to such climate extremes is therefore important to understand (Walsh et al., 2019), especially given the recent discovery that drosophilid species distributions are determined primarily by male thermal fertility limits, and not viability or lethal thresholds (Parratt et al., 2020).

Data Availability

All raw data and code in support of this study are available to download at https://doi.org/10.5061/dryad.

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Appendix

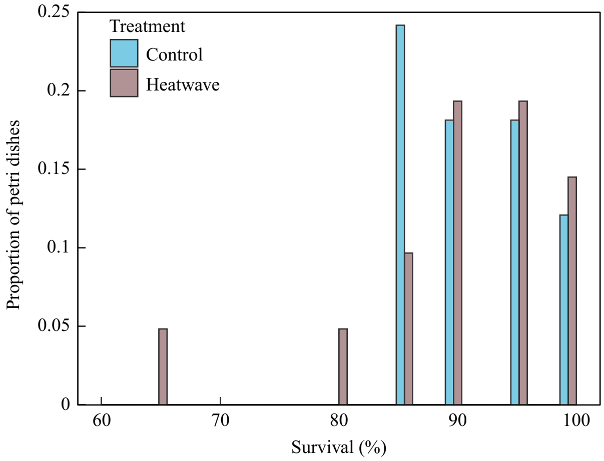

Figure A1. Survival of males, from collection at pupal stage to after the heatwave or control treatment. Pupae were kept in groups of 20 in 6 cm petri dishes with 15 ml of fodder (15 groups for heatwave treatment, 12 for control treatment).

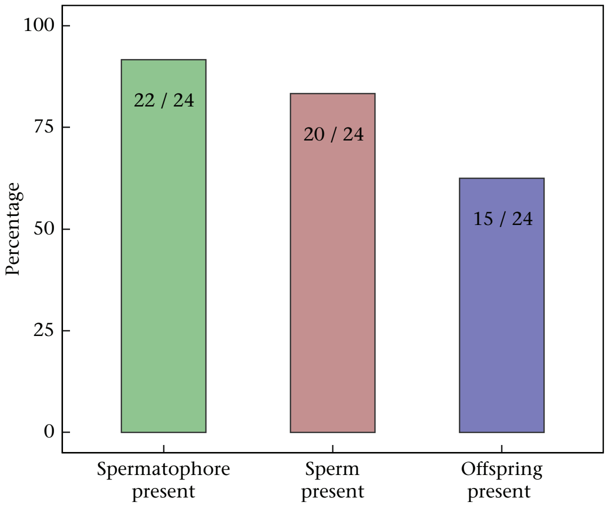

Figure A2. Percentage of females that after a 24 h mating opportunity with a heatwave-exposed male were found to have a spermatophore and/or sperm present in the bursa copulatrix and the spermatophore, respectively. Mating vials were also incubated to check for the presence of offspring 35 days later. Numbers represent numbers of females.

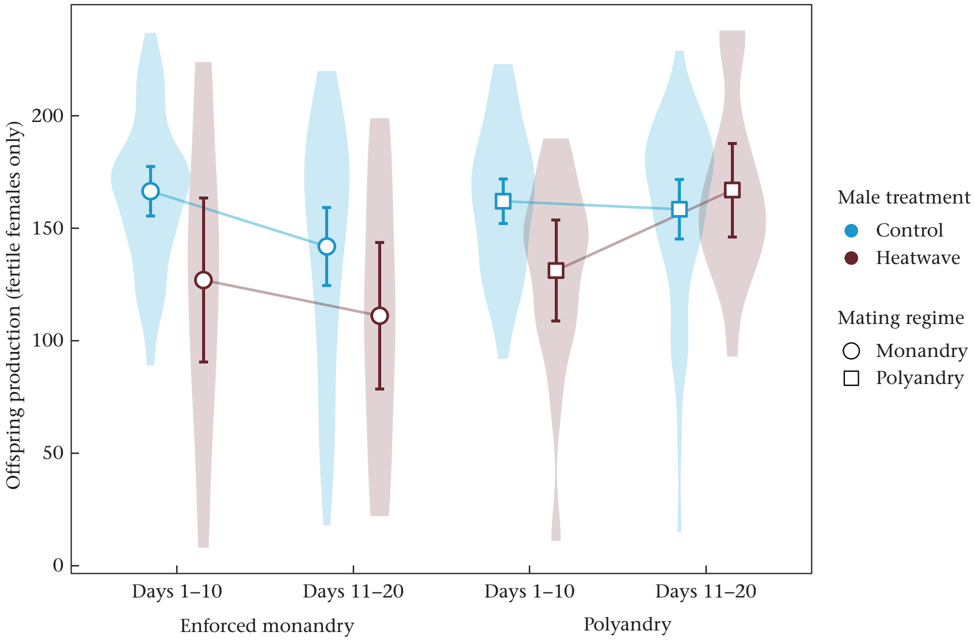

Figure A3. Offspring production of females first mated to control males or heatwave-exposed males and then after 10 days either not allowed to remate (enforced monandry) or allowed to remate (polyandry). Results are shown for females with nonzero reproductive output (see Fig. 5). Here, we excluded females that had failed to produce any offspring in one of their laying vials. Large symbols and error bars show means and approximate 95% confidence intervals. Raw data are represented by violin plots.