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Elevated temperature changes female costs and benefits of reproduction

Vera M. Grazer · Oliver Y. Martin

Abstract Despite obvious benefits, reproduction also imposes severe costs on females. Such costs and benefits are highly sensitive to environmental factors. Rapidly changing conditions may thus disturb a finely poised balance between the two and pose a challenge to natural populations. A more complete understanding of reproduction and population fitness across different environments is, hence, crucial. In particular, sexual selection could either be beneficial or detrimental when conditions change abruptly. Here *Tribolium castaneum* females were subjected to mating treatments with or without sexual selection (virginity, monogamy, polyandry) replicated at standard versus elevated temperatures. We found a substantial survival cost of reproduction at the standard, but not at the elevated temperature. Reproductive success was similar across mating treatments at the standard temperature, but at elevated temperature we detected a significant benefit of polyandry compared to monogamy. These findings indicate that environmental heterogeneity can strongly influence the balance between costs and benefits when sexual selection is allowed to act. Furthermore, reproduction may be critically affected by changes in temperature with potentially profound consequences for population fitness.

Keywords Sexual selection · Mating costs · *Tribolium* · Environmental change · Fitness

Introduction

Precise knowledge of the major costs and benefits of reproduction is necessary to understand how sexual selection affects population productivity and fitness under given ecological conditions. Reproduction is certainly a costly endeavour for females (Partridge and Farquhar 1981; Blanckenhorn et al. 2002; Martin and Hosken 2004). Possible costs may

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arise via transmission of disease (Hurst et al. 1995), loss of energy (Watson et al. 1998), harmful seminal fluid proteins (Johnstone and Keller 2000) or mechanical damage caused during copulation (Crudgington and Siva-Jothy 2000). Overall, mating more often than necessary can, hence, be very costly for females and potentially decrease survival. Nevertheless, most female insects mate polyandrously, thus costs incurred via increased sexual activity need to be traded-off against potential benefits of polyandry (reviewed in Jennions and Petrie 2000; Zeh and Zeh 2001; Hosken and Stockley 2003). Under constant conditions, it might be expected that on average the costs of reproduction are offset by benefits of increased reproductive success due to competition or choice.

Few studies have attempted to simultaneously measure both the costs and benefits of reproduction (Fricke et al. 2009), likely because of the associated experimental difficulties. Experimental evolution experiments have succeeded in detecting hidden mating costs and exposing sexual conflicts in various insect systems (Rice 1996; Holland and Rice 1999; Hosken et al. 2001; Martin and Hosken 2003a, b; Arnqvist and Rowe 2005; Wigby and Chapman 2005). These findings indicate that it may be necessary to alter conditions experimentally in order to reveal and thus be able to measure all costs and benefits. There is, actually, accumulating evidence showing that warming affects reproductive and sexually selected traits (Chihrane and Lauge 1994; Hughes 2000; West and Packer 2002). Therefore, mating costs and benefits are likely sensitive to the environmental context, yet so far this has been neglected, at least empirically (Candolin and Heuschele 2008; Fricke et al. 2009).

A considerable part of reproductive costs for polyandrous females probably occur via increased energetic demands compared to monogamous females (Watson et al. 1998; Crudgington and Siva-Jothy 2000; Blanckenhorn et al. 2002; Martin and Hosken 2004). Rapid change of environmental conditions at reproduction may challenge the female if additional energy expenditure is required to deal with physiological stress or acclimatisation to new conditions (Angilletta 2009). In the short-term, energetic costs may accumulate. In addition, it has been shown theoretically that under rapid environmental change sexually selected traits are unable to track ecological optima (Tanaka 1996). This suggests that when sexual selection is involved, such as in polyandrous mating, reproducing females may be further burdened with costs. Following rapid environmental change female fitness, thus, may likely deteriorate. Overall, it is conceivable that the degree and speed of environmental change will determine the magnitude and direction of change in fitness. Therefore, shifts in the cost-benefit balance of reproduction, potentially towards more costs, may be expected.

In 2007, the IPCC predicted that increasing global mean temperatures will continually change the conditions of formerly stable niches (Meehl et al. 2007). Due to these changes, many species will not be able to reside in habitats to which they are accustomed (Chevin et al. 2010). Maintaining high fitness in such a situation is, hence, absolutely crucial for the survival of populations and species. In insects, effects of environmental change are already becoming apparent (Bale et al. 2002; Menendez 2007). On the one hand, insects may profit from warmer temperatures leading to faster reproduction and dispersal (Hughes 2000). On the other hand, insects are at risk from global warming, since they mainly occupy heavily affected terrestrial habitats (Maes et al. 2010).

Here we use *Tribolium castaneum*, a highly promiscuous beetle (Pai and Bernasconi 2008), to test whether the potential cost-benefit balance of reproduction prevailing under constant conditions is shifted towards more fitness costs if temperature is elevated abruptly (Tanaka 1996). To achieve this, we performed mating experiments at the standard temperature of our lab strain (maintained at 30°C for >30y.) and at an elevated temperature

(34°C). We experimentally monitored female reproductive success and survival under both monogamous and polyandrous scenarios in order to investigate possible advantages and disadvantages of sexual selection. The experiment was run over a period of 10 weeks, which allowed us to detect both short and long-term effects and to assess fitness costs and benefits more accurately. We had the following specific predictions. (i) Increasing temperature is stressful and will reduce female survival and reproductive success. (ii) Polyandrous mating compared to monogamy imposes costs, which reduce female survival. (iii) Polyandry (i.e. the typical mating situation for *Tribolium*) leads to higher reproductive success than monogamy. (iv) Elevating temperature may increase the benefits of polyandry, via the presence of sexual selection.

Materials and methods

Mating experiment

Tribolium castaneum beetles used in this experiment were derived from the Georgia 1 stock (see Michalczyk et al. 2010, 2011) obtained from Richard Beeman at USDA. This stock has been constantly maintained at 30°C and in large numbers at USDA and subsequently in our lab (i.e. over 30 years, equivalent to ca. 350 beetle generations). Beetles were collected as pupae to separate the sexes. Pupae were maintained on our standard flour-yeast mix (organic white flour with 10% yeast sterilised at 80°C for ca. 24 h) for hatching and maturation. All adults were at least 1 week post-emergence when the experiment was started. Two days before the experiment females were marked on the thorax with "Queen marker" pen (Bienen-Meier, Künten, Switzerland).

Single females were housed in 5 cm diameter Petri-dishes containing flour (15 ml ≈ 7 g) topped with a layer of Soy-flakes. Females were randomly assigned to one of four mating treatments: no male (virgins), one male (monogamy), six males (polyandry) and twelve males (polyandry). Polyandry, allowing for the effect of sexual selection, was thus investigated in two treatments (6 and 12 males), since potential costs might be cumulative and increase in strength with more mates (see Fig. 1 in Michalczyk et al. 2011). Each mating treatment was replicated at both standard rearing temperature (30°C) and elevated temperature (34°C). Hence, the experiment combined mating treatment and temperature, and each of these combinations consisted of 30 females. During the whole experiment, females were kept constantly at either 30 or 34°C. The mating phase of the experiment was 1 week during which the females were allowed to copulate freely with the available number of mates according to treatment. This set-up was chosen for the following reasons: (i) mating groups enable precopulatory sexual selection, which would not be possible with controlled sequential matings. (ii) During the single week of exposure to mates very few males die, keeping the intended female/male ratio constant. (iii) Keeping males in the experiment in different numbers across treatments is not feasible, as males cannibalize eggs and disturb oviposition. (iv) *Tribolium* can store sperm (Sokoloff 1972) and already after few copulations the spermatheca should be filled to capacity (Lewis and Jutkiewicz 1998). We can, therefore, assume that females stored a full load of sperm during this standardized period of access to males. Because of these reasons, our design enabled us to initially allow or disallow sexual selection for a standard period, but thereafter to assess declining reproductive success over time (as sperm stores are used up) without male interference and harassment. Crucially, this also allows us to pick up any short or long-term fertility benefits of access to multiple versus single mates.
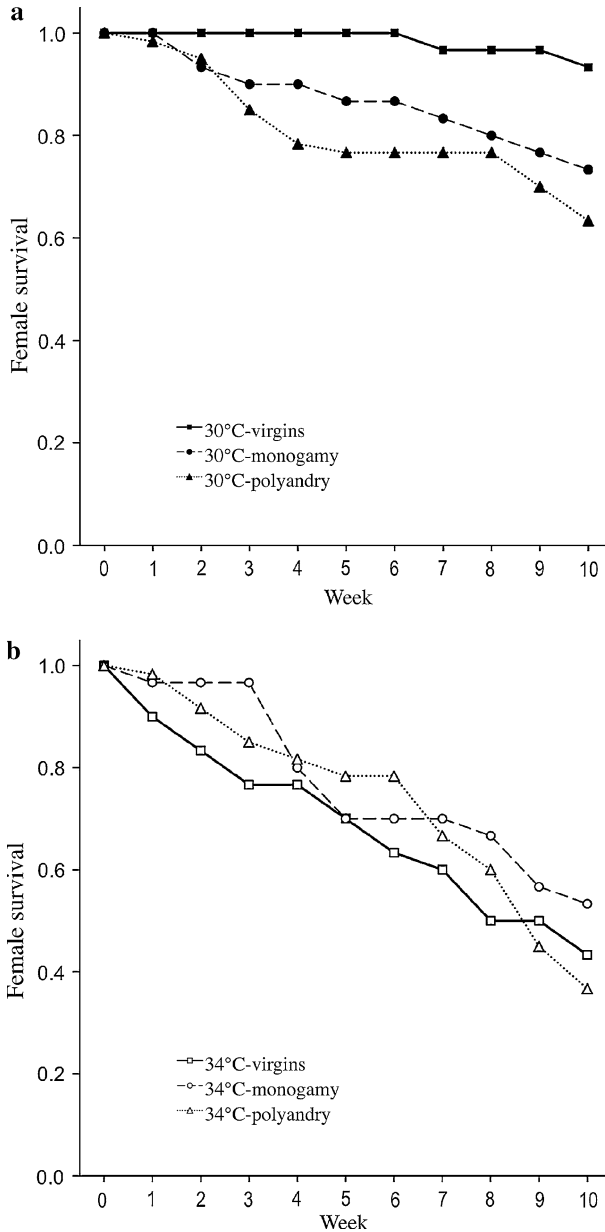
Fig. 1 Proportion of females surviving **a** at standard temperature (30°C) and **b** at elevated temperature (34°C) as a function of time and mating regime (*Initial sample sizes* virgins = 30, monogamy = 30, polyandry [6 or 12 mates] = 60]

After 1 week of mating, all males were removed from the experiment. If the marked female was alive, she was transferred to a new Petri-dish with fresh flour, otherwise the time of death was noted. Females were then kept in this way for an additional 9 weeks to track offspring production rates over time. During this time, females were checked weekly

to determine their time of death and transfer them to new flour. There was no need to transfer virgins, as flour is provided in great excess. All Petri-dishes containing developing offspring were incubated for 3 weeks according to the temperature treatment of the mother. We thus counted larvae and not eggs as our fitness response, as it is not feasible to distinguish fertilized from unfertilized eggs. Furthermore, we aimed to observe temperature effects including effects on egg and larval survival. To do so, offspring had to be counted before larval competition influences offspring survival. All offspring were counted per week, resulting in our measure of total reproductive success over the period of 10 weeks.

Statistical analysis

Female survival was analyzed with Cox survival analysis using parametric regression. Total reproductive success was analyzed with general linear models (GLM) incorporating survival as a covariate. In case of unbalanced data the model testing was performed using type III sums of squares. Residuals of the models were checked with the Shapiro–Wilk test and were normally distributed. Number of offspring per week was analyzed with a general linear mixed model for repeated measures. The residuals were checked within the randomized unit 'week', which fulfilled the model assumptions. All statistical analyses were conducted with the R statistical package (R Development Core Team 2009).

Results

Survival

Comparing mean survival between the two polyandrous mating treatments with 6 versus 12 males showed no effect of male number (6 m at 30°C: 9.2 ± 0.6 weeks, 12 m at 30°C: 8.7 ± 0.6 weeks/6 m at 34°C: 8.2 ± 0.6 weeks, 12 m at 34°C: 8.2 ± 0.6 weeks). Cox regression showed that beyond a significant temperature effect ($\chi_1^2 = 6.05$, $P = 0.014$, $n = 120$) neither the main effect of male number ($\chi_1^2 = 0.65$, $P = 0.421$, $n = 120$) nor the interaction between male number and temperature ($\chi_1^2 = 2.39$, $P = 0.122$, $n = 120$) were significant. Because of this finding, the females of the two polyandrous treatments were combined and the data was analysed between the mating regimes virgin, monogamy and polyandry.

Female survival was significantly affected by the interaction of temperature and mating regime ($\chi_1^2 = 8.77$, $P = 0.012$, $n = 240$). Mated females died earlier than virgins at the standard, but not at the elevated temperature (Fig. 1). The temperature effect on survival was highly significant ($\chi_1^2 = 21.71$, $P < 0.001$, $n = 240$), but the main effect of mating regime was not significant ($\chi_1^2 = 5.12$, $P = 0.077$, $n = 240$). When the virgin groups were excluded to allow direct comparison of monogamous versus polyandrous mating, only the main effect of the temperature regime remained significant (temperature: $\chi_1^2 = 8.73$, $P = 0.003$, $n = 180$; mating: $\chi_1^2 = 2.72$, $P = 0.099$; temperature $\times$ mating: $\chi_1^2 = 0.03$, $P = 0.872$).

Total reproductive success

Mean total reproductive success in the two polyandrous treatments with 6 or 12 males was very similar (6 m at 30°C: 184 ± 21, 12 m at 30°C: 178 ± 15/6 m at 34°C: 225 ± 20,

12 m at 34°C: 225 ± 23). ANCOVA using male number (6, 12), temperature (standard, elevated) and their interaction as explanatory variables and survival as covariate showed no significant difference between these treatments regarding the interaction of male number with temperature ($F_{1,115} = 0.000$, $P = 0.997$) or the main effect of male number ($F_{1,115} = 0.03$, $P = 0.863$). The largest proportion of variability in total reproductive success was explained by female survival ($F_{1,115} = 100.16$, $P < 0.001$), and temperature also had a significant influence ($F_{1,115} = 8.95$, $P = 0.003$).

Since the two polyandrous groups were not different, they were combined to compare the effect of no sexual selection (monogamy) versus sexual selection (polyandry) on total reproductive success. Thus, sexual selection (monogamy, polyandry), temperature (standard, elevated) and their interaction were included in the model and survival as a covariate. There was no interaction of the presence of sexual selection and temperature; however, total reproductive success was significantly affected by the presence or absence of sexual selection, temperature and female survival (Table 1). In addition, the same analysis was performed excluding offspring produced during the first week of the experiment, where males had been present. The results were qualitatively the same, as sexual selection, temperature and female survival remained significant at the 0.01-significance level. At the standard temperature, polyandrous females had a similar amount of offspring than monogamous females. However, at the elevated temperature, polyandrous females produced more offspring (Fig. 2).

Offspring per week

The number of offspring that females produced during the experiment was variable (Fig. 3). In particular, the largest numbers of offspring were produced during the first 6 weeks, and this difference was more marked at the elevated temperature (Fig. 3). During the last 4 weeks of the experiment, females at both temperatures produced fewer offspring than before, although the females at standard temperature produced offspring for longer (Fig. 3). Note also, at the standard temperature where monogamous and polyandrous females produced similar amounts of offspring, it is clearly visible that both groups produced offspring for a comparable period of time. This finding indicates that our assumption that all females were able to store a full load of sperm in the spermatheca was fulfilled (Fig. 3a).

First, we analyzed offspring production per week only between the polyandrous groups using male number (6 males, 12 males) and temperature (standard, elevated) as fixed factors and week as a random factor and including all possible interactions. Male number and all interactions including this factor were again not significant (male number: $F_{1,116} = 0.10$, $P = 0.757$; male number $\times$ temp.: $F_{1,116} = 0.00$, $P = 0.962$;

Table 1 Analysis of covariance of the effects of sexual selection (monogamy; polyandry), temperature (30, 34°C) and their interaction on total reproductive success (correcting for female survival)

| Source	DF	F	P
Sexual selection	1	6.83	0.010
Temperature	1	10.25	0.002
Survival	1	104.20	<.0001
Sexual selection × temperature	1	2.84	0.094
Residuals	175		
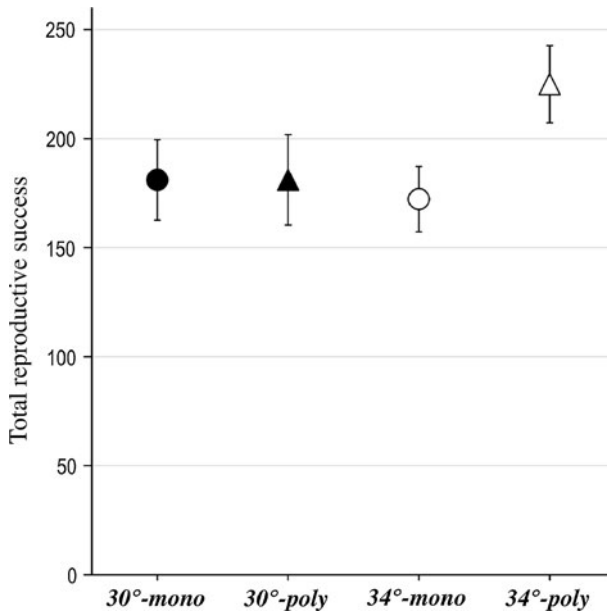
Fig. 2 Total female reproductive success (mean ± 1 SE) at standard (30°C) or elevated (34°C) temperature over 10 weeks after mating either monogamously (mono = 1 male, no sexual selection, $n = 30$), or polyandrously (poly = 6 or 12 males, with sexual selection, $n = 60$)

male number × week: $F_{1,848} = 0.55$, $P = 0.458$; male number × temp. × week: $F_{1,848} = 0.05$, $P = 0.824$). Only those factors unrelated to male number significantly affected offspring production in these groups (temperature: $F_{1,116} = 13.09$, $P < 0.001$; week: $F_{1,848} = 460.82$, $P < 0.001$; temp. × week: $F_{1,848} = 45.45$, $P < 0.001$).

Females from the polyandrous groups were, hence, again combined to compare the effect of monogamous versus polyandrous mating (=factor sexual selection). The results are summarized in Table 2 showing that the interaction of sexual selection and temperature was marginally not significant but that sexual selection and temperature had significant effects on offspring production (Table 2; Fig. 3). When non-significant interactions were removed from this model, the results remained qualitatively the same. Additionally, this was also the case when the same analysis was performed excluding the offspring produced during the first week.

Discussion

Here we present responses in female survival and reproductive success to elevated temperature in presence versus absence of sexual selection. Interestingly, we not only document a quantitative change but a qualitatively totally different outcome under different environmental conditions. There was a substantial survival cost of reproduction at the standard, but not at elevated temperature. In addition, total reproductive success was similar across mating treatments at standard temperature, whereas at elevated temperature access to multiple mates was beneficial.
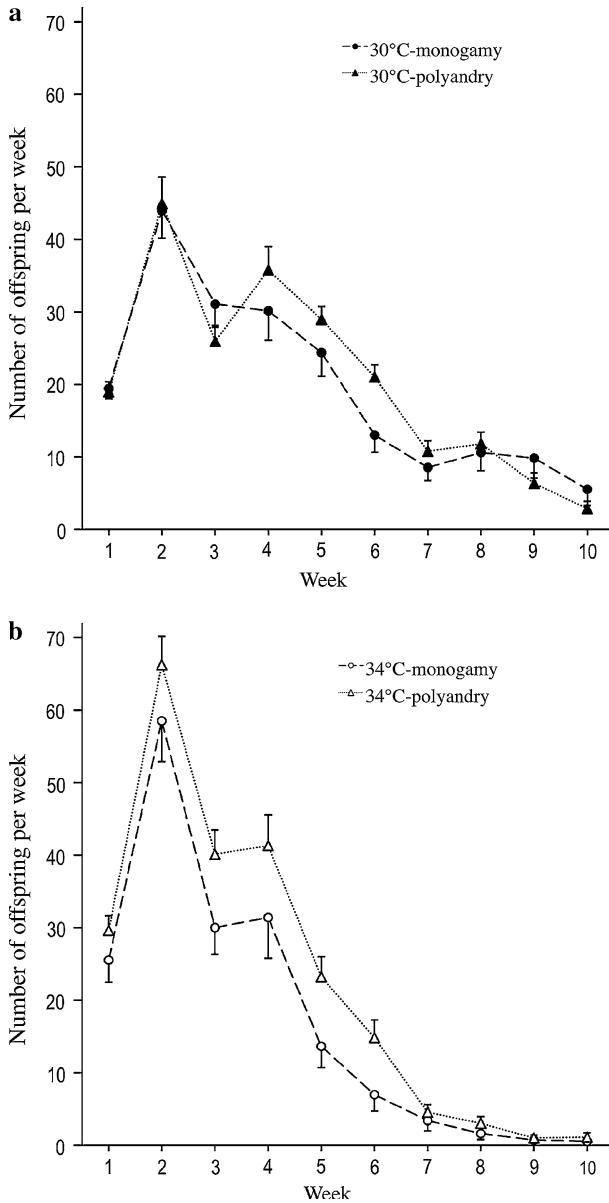
Fig. 3 Number of offspring produced per week **a** at standard (30°C) or **b** at elevated (34°C) temperature (monogamous females $n = 30$, polyandrous females (6 or 12 mates) $n = 60$; *error bars* represent 1 SE)

It is now clear that environmental change rapidly alters ecological niches of many species, for example, many more species will have to cope with increasing temperatures in the near future (Thomas et al. 2004; Meehl et al. 2007). Clearly, reproductive success and survival are eminently important factors determining population demographics and viability (Reed 2008; Williams et al. 2008). In this respect, our findings underline how strongly fitness traits can change with the environment. This may of course have severe

Table 2 General linear mixed effects model for number of offspring per week produced by females where sexual selection was enabled (polyandry) or disabled (monogamy) at two temperatures (30, 34°C)

Source	Numerator (DF)	Denominator (DF)	F	P
Sexual selection	1	176	4.49	0.035
Temperature	1	176	10.50	0.001
Week	1	1,295	713.05	<.0001
Sexual selection × temp.	1	176	3.19	0.076
Sexual selection × week	1	1,295	2.42	0.120
Temp. × week	1	1,295	57.69	<.0001
Sexual selection × temp. × week	1	1,295	2.40	0.122

impacts on population fitness especially if the cost-benefit balance of reproduction is shifted from one direction to the other. In theoretical terms Tanaka (1996) studied a sexually selected trait under environmental change and predicted a shift towards more reproductive costs. Our results on survival suggest that costs may indeed change and that, in principle, predictions such as those made by Tanaka could well apply. Here, however, it was not the case that environmental change via elevated temperature had a negative impact on population fitness as we had generally expected. On the contrary, neither monogamous nor polyandrous females suffered from costs of reproduction under changed conditions.

The *Tribolium* strain used is well adapted to the standard temperature (>30 years at 30°C), but can reproduce at up to 38°C (personal observation). Female reproductive success and survival changed substantially after increasing the environmental temperature by only 4°C. Consistent with our prediction (i), virgins survived better in the environment of origin, whereas the elevated temperature led to a survival cost. In *T. castaneum* a range of proteins are expressed in a heat-shock response (Mahroof et al. 2005). Increased protein production is likely costly, which may have reduced female survival. Additionally, at elevated temperature females will likely have suffered from increased water loss (Hadley 1994). This may also have increased the costs of survival if females were unable to replenish enough water.

At the standard temperature mated females incurred a cost of mating through a survival reduction compared to virgins. This cost, however, was not observed at elevated temperature. During copulation males transfer seminal fluid, which females may store and use as a nutrient or water source. Theoretically, ejaculates may help to deal with temperature and desiccation stress (Poiani 2006; Edvardsson 2007). This effect has indeed been described in various arthropod taxa (beetle: Fox 1993; moth: Svard and Mcneil 1994; cricket: Ivy et al. 1999; butterfly: Wiklund et al. 2001). It may be possible, therefore, that mated females were able to partly compensate for the negative effects of elevated temperature and potentially reproduction on survival. In fact, this finding seems robust, as virgins had consistently lower survival than mated females from the first until the 8th week of the experiment. This corroborates the notion that females may have benefitted from mating at elevated temperature.

Alternatively, Cohet and David (1976) investigated deleterious effects of copulation in *Drosophila* females as a function of temperature, and showed that male harmfulness and female survival were highly dependent on male growth temperature. In accordance with our study, female survival costs were higher at intermediate temperatures compared to less survival costs at higher temperature. Mating costs thus depend on the environment, and

rather than being obscured (via compensation, see above), may simply cease to occur in a different context.

Polyandry was not associated with any survival costs for females at either temperature, so we cannot confirm prediction (ii). This finding indicates that costs of multiple mating may be minor in *T. castaneum*. This notion is in agreement with previous work on this species: females are very willing to mate, and the number of mates has no effect on female survival (Pai and Yan 2003). Additionally, using an experimental evolution approach and the same strain, it was found that females evolving under male-biased sex ratios coped well with exposure to large numbers of mates (Michalczyk et al. 2011). Together, these findings suggest that, under standard conditions, *T. castaneum* females may be well adapted to handling negative effects of copulation.

Focussing on reproductive success rather than survival, the present and a previous study on *T. castaneum* did not detect fitness benefits (or costs) of polyandry at standard temperature (Pai and Yan 2003). This contradicts prediction (iii). Interestingly, though, we do find higher reproductive success at the elevated temperature, which supports prediction (iv). Many studies suggest that females of this species are able to influence paternity by (cryptic) female choice, for instance by expelling spermatophores or differently processing sperm for storage (Qazi et al. 1998; Nilsson et al. 2003; Fedina and Lewis 2004; Lewis et al. 2005; Fedina and Lewis 2007). Spermathecal gland secretions may also chemically influence the environment that sperm encounter (Fedina and Lewis 2008). Furthermore, in a study by Ward et al. (2002) eggs of yellow dung flies had different paternal genotypes across shaded and sunlit areas, which suggests that postcopulatory female choice according to environmental cues may be possible. Similarly, here, sexual selection or bet-hedging (Fox and Rauter 2003) could have biased paternity in offspring of polyandrous mothers at elevated temperature, as polyandry increases the chance that mates (or sperm) which perform better at elevated temperature are present (Pizzari and Parker 2009).

In contrast with prediction (i), elevated temperature led to higher total reproductive success on average. Females maintained at elevated temperature produced larger numbers of offspring early on (weeks 1–4) but productivity seemed to decline more steeply than under the standard temperature (see Fig. 3). This pattern may be explained with the terminal investment hypothesis (Clutton-Brock 1984). Females experiencing temperature stress might increase their investment into reproduction in response to a potential threat to their survival. In the present experiment temperature stress was clearly present as reflected in shorter female survival at the elevated temperature. Indeed, similar mechanisms have already been documented in other species including the closely related *Tenebrio molitor* (Sadd et al. 2006). However, this possibility does not seem sufficient to explain our results, especially as a previous study finds no evidence suggestive of terminal investment in the same strain of *T. castaneum* (Morrow et al. 2003). On closer inspection, this effect seems to be driven by polyandrous females having superior total reproductive success at elevated temperature. Since we tracked female reproductive success over a period of 10 weeks, we were able to observe that monogamous females could successfully produce offspring for as long as polyandrous females. This rules out the possibility that monogamous females simply had fewer sperm available.

To conclude, elevated environmental temperature led to the disappearance of a reproductive cost on survival and the appearance of a fitness benefit for polyandrous females. This is a strong signal that the cost-benefit balance of reproduction is highly temperature sensitive. We demonstrate that by increasing temperature by 4°C the balance can be shifted drastically in a new direction, in this case towards higher fitness. This study also indicates how we may explain different outcomes of environmental change across species. Species-

specific factors, such as the contrasting intensities of sexual selection implemented in the present study, may determine in which direction the cost-benefit balance is shifted. In a general and applied sense, there is an urgent need to perform more experiments under contrasting environmental conditions such as implemented here or in Hunt et al. (2004) for nutritional environment. Indeed, interest in incorporating the role of the environment in the study of sexual selection is growing steadily (e.g. Fricke et al. 2009; Ingleby et al. 2010; Narraway et al. 2010). More precise knowledge regarding how central fitness traits, such as survival and reproductive success, change under different conditions would further provide important insights to understanding population sensitivity.

References

Angilletta MJ (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford

Arnqvist G, Rowe L (2005) Sexual conflict. Princeton University Press, Princeton

Bale JS, Masters GJ, Hodkinson ID et al (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Glob Change Biol 8:1–16

Blanckenhorn WU, Hosken DJ, Martin OY et al (2002) The costs of copulating in the dung fly *Sepsis cynipsea*. Behav Ecol 13:353–358

Candolin U, Heuschele J (2008) Is sexual selection beneficial during adaptation to environmental change? TREE 23:446–452

Chevin LM, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol 8:1–8

Chihrane J, Lauge G (1994) Effects of high-temperature shocks on male germinal cells of *Trichogramma brassicae* (Hymenoptera, Trichogrammatidae). Entomophaga 39:11–20

Clutton-Brock TH (1984) Reproductive effort and terminal investment in iteroparous animals. Am Nat 123:212–229

Cohet YA, David JR (1976) Deleterious effects of copulation in *Drosophila* females as a function of growth temperature of both sexes. Experientia 32:696–697

Crudgington HS, Siva-Jothy MT (2000) Genital damage, kicking and early death—the battle of the sexes takes a sinister turn in the bean weevil. Nature 407:855–856

Edvardsson M (2007) Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. Anim Behav 74:183–188

Fedina TY, Lewis SM (2004) Female influence over offspring paternity in the red flour beetle *Tribolium castaneum*. Proc Roy Soc Lond B 271:1393–1399

Fedina TY, Lewis SM (2007) Female mate choice across mating stages and between sequential mates in flour beetles. J Evol Biol 20:2138–2143

Fedina TY, Lewis SM (2008) An integrative view of sexual selection in *Tribolium* flour beetles. Biol Rev 83:151–171

Fox CW (1993) Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera, Bruchidae). Funct Ecol 7:203–208

Fox CW, Rauter CM (2003) Bet-hedging and the evolution of multiple mating. Evol Ecol Res 5:273–286

Fricke C, Perry J, Chapman T et al (2009) The conditional economics of sexual conflict. Biol Lett 5:671–674

Hadley NF (1994) Water relations of terrestrial arthropods. Academic Press, San Diego

Holland B, Rice WR (1999) Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. PNAS 96:5083–5088

Hosken DJ, Stockley P (2003) Benefits of polyandry: a life history perspective. Evol Biol 33:173–194

Hosken DJ, Garner TWJ, Ward PI (2001) Sexual conflict selects for male and female reproductive characters. Curr Biol 11:489–493

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? TREE 15:56–61

Hunt J, Brooks R, Jennions MD et al (2004) High-quality male field crickets invest heavily in sexual display but die young. Nature 432:1024–1027

Hurst GDD, Sharpe RG, Broomfield AH et al (1995) Sexually-transmitted disease in a promiscuous insect, *Adalia bipunctata*. Ecol Entomol 20:230–236

Ingleby FC, Hunt J, Hosken DJ (2010) The role of genotype-by-environment interactions in sexual selection. J Evol Biol 23:2031–2045

Ivy TM, Johnson JC, Sakaluk SK (1999) Hydration benefits to courtship feeding in crickets. Proc Roy Soc Lond B 266:1523–1527

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Johnstone RA, Keller L (2000) How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. Am Nat 156:368–377

Lewis SM, Jutkiewicz E (1998) Sperm precedence and sperm storage in multiply mated red flour beetles. Behav Ecol Sociobiol 43:365–369

Lewis SM, Kobel A, Fedina T et al (2005) Sperm stratification and paternity success in red flour beetles. Physiol Entomol 30:303–307

Maes D, Titeux N, Hortal J et al (2010) Predicted insect diversity declines under climate change in an already impoverished region. J Ins Conserv 14:485–498

Mahroof R, Zhu KY, Neven L et al (2005) Expression patterns of three heat shock protein 70 genes among developmental stages of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Comp Biochem Physiol A 141:247–256

Martin OY, Hosken DJ (2003a) Costs and benefits of evolving under experimentally enforced polyandry or monogamy. Evolution 57:2765–2772

Martin OY, Hosken DJ (2003b) The evolution of reproductive isolation through sexual conflict. Nature 423:979–982

Martin OY, Hosken DJ (2004) Copulation reduces male but not female longevity in *Saltella sphondylli* (Diptera: Sepsidae). J Evol Biol 17:357–362

Meehl GA, Stocker TF, Collins WD et al (2007) Global climate projections. In: Solomon S, Qin D, Manning M et al (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, pp 747–845

Menendez R (2007) How are insects responding to global warming? Tijdschrift voor Entomol 150:355–365

Michalczyk L, Martin OY, Millard AL et al (2010) Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. Proc Roy Soc Lond B 277:3483–3491

Michalczyk L, Millard AL, Martin OY et al (2011) Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. Evolution 65:713–724

Morrow EH, Arnqvist G, Pitnick S (2003) Adaptation versus pleiotropy: why do males harm their mates? Behav Ecol 14:802–806

Narraway C, Hunt J, Wedell N et al (2010) Genotype-by-environment interactions for female preferences. J Evol Biol 23:2550–2557

Nilsson T, Fricke C, Arnqvist G (2003) The effects of male and female genotype on variance in male fertilization success in the red flour beetle (*Tribolium castaneum*). Behav Ecol Sociobiol 53:227–233

Pai A, Bernasconi G (2008) Polyandry and female control: the red flour beetle *Tribolium castaneum* as a case study. J Exp Zool (Mol Dev Evol) 310B:148–159

Pai A, Yan GY (2003) Rapid female multiple mating in red flour beetles (*Tribolium castaneum*). Can J Zool 81:888–896

Partridge L, Farquhar M (1981) Sexual activity reduces lifespan of male fruitflies. Nature 294:580–582

Pizzari T, Parker GA (2009) Sperm competition and sperm phenotype. In: Birkhead TR, Hosken DJ, Pitnick S (eds) Sperm biology: an evolutionary perspective. Elsevier Academic Press, Burlington, pp 207–245

Poiani A (2006) Complexity of seminal fluid: a review. Behav Ecol Sociobiol 60:289–310

Qazi MCB, Aprille JR, Lewis SM (1998) Female role in sperm storage in the red flour beetle, *Tribolium castaneum*. Comp Biochem Physiol A 120:641–647

Reed DH (2008) Effects of population size on population viability: from mutation to environmental catastrophes. In: Carroll SP, Fox CW (eds) Conservation biology: evolution in action. Oxford University Press, Oxford, pp 16–34

Rice WR (1996) Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 381:232–234

Sadd B, Holman L, Armitage H et al (2006) Modulation of sexual signalling by immune challenged male mealworm beetles (*Tenebrio molitor* L.): evidence for terminal investment and dishonesty. J Evol Biol 19:321–325

Sokoloff A (1972) The biology of *Tribolium* with special emphasis on genetic aspects. Oxford University Press, Oxford

Svard L, Mcneil JN (1994) Female benefit, male risk—polyandry in the true armyworm *Pseudaletia unipuncta*. Behav Ecol Sociobiol 35:319–326

Tanaka Y (1996) Sexual selection enhances population extinction in a changing environment. J Theor Biol 180:197–206

Thomas CD, Cameron A, Green RE et al (2004) Extinction risk from climate change. Nature 427:145–148

Ward PI, Vonwil J, Scholte EJ et al (2002) Field experiments on the distributions of eggs of different phosphoglucomutase (PGM) genotypes in the yellow dung fly *Scathophaga stercoraria* (L.). Mol Ecol 11:1781–1785

Watson PJ, Arnqvist G, Stallmann RR (1998) Sexual conflict and the energetic costs of mating and mate choice in water striders. Am Nat 151:46–58

West PM, Packer C (2002) Sexual selection, temperature, and the lion's mane. Science 297:1339–1343

Wigby S, Chapman T (2005) Sex peptide causes mating costs in female *Drosophila melanogaster*. Curr Biol 15:316–321

Wiklund C, Karlsson B, Leimar O (2001) Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. Proc Roy Soc Lond B 268:1661–1667

Williams SE, Shoo LP, Isaac JL et al (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biol 6:2621–2626

Zeh JA, Zeh DW (2001) Reproductive mode and the genetic benefits of polyandry. Anim Behav 61:1051–1063