

1 **Polyandry promotes successful colonisation in novel thermal envi-**
2 **ronments**

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Abstract

It is increasingly recognised that polyandry and sexual selection may enable successful colonisation of novel environments. In addition to the benefits provided by sexual selection, polyandry can promote successful colonisation through minimising the impact of mating with unsuitable males, and by reducing levels of inbreeding in founder populations. Here we show that polyandry provides substantial benefits in the colonisation of novel, and harsh, thermal environments. Using colonisation experiments with the model beetle *Tribolium castaneum*, we founded populations at increased temperature using either singly or doubly mated females, and followed population dynamics for ten generations. We found that extinction rates were significantly lower (9%) in polyandrous-founded populations compared to monogamous-founded populations (34%). Further, we found significant and consistently higher population sizes in polyandrous compared to monogamous-founded populations. Importantly, we identified distinct short-term and delayed benefits of multiple mating for population persistence and fitness, suggesting that polyandry acts to both buffer against mating with infertile males, and reduce inbreeding depression. Our results therefore suggest that polyandry provides substantial benefits for colonising populations, on top of those provided by sexual selection.

Key words: colonisation, extinction, population dynamics, sexual selection, *Tribolium*

23 Introduction

24 Global warming, when combined with other anthropogenic pressures, is placing a range of ecological and evo-
25 lutionary stresses on wild populations [1,2]. In particular, a combination of habitat clearance and changing
26 temperatures will result in populations simultaneously going through bottlenecks, while needing to respond
27 to novel habitats and thermal regimes [3]. Understanding the behavioural, ecological, and genetic processes
28 by which bottlenecked populations respond to novel thermal environments is therefore of relevance to un-
29 derstanding - and ultimately predicting - organismal responses to global warming [4]. Disentangling these
30 processes requires a combination of laboratory and field research at a wide range of organisational levels [5].

31 One way that populations may be buffered against demographic and environmental change is through mul-
32 tiple mating, or polyandry [6,7]. In addition to the direct benefits that polyandry may provide to females
33 and their offspring (e.g. increased resources from males) [8], multiple mating can provide genetic benefits [9].
34 Polyandry facilitates sexual selection, in which females may provide genetic benefits to their descendants
35 through biasing paternity towards specific males [10]. There are multiple mechanisms by which sexual selec-
36 tion may improve population fitness [11], including offspring inheriting either “good” or “compatible” genes
37 as a result of female mate-choice [12], or through mate choice for increased genetic diversity in offspring,
38 which may increase adaptability to fluctuating environments [13].

39 Polyandry may also provide benefits to individuals and populations even in the absence of sexual selection.
40 The best studied mechanism through which this may occur is bet-hedging [14]. If mate-choice is unreliable
41 and there is variance in male fitness, indiscriminate mating with more than one male may be an effective
42 strategy. It is still debated whether the fitness gains derived from bet-hedging are sufficient to drive the
43 evolution of polyandry [15]. However, bet-hedging may play an important role in the dynamics of small
44 colonising populations, in which the consequences of mating with a single, low-quality male are expected to
45 be particularly severe [16].

46 Finally, polyandry may enable colonisation of new environments through short-term reductions in inbreed-
47 ing. Cornell and Tregenza [17] developed a model showing that because offspring of polyandrous females
48 contain half-sibs, inbreeding depression in future generations will be reduced, leading to greater probability of
49 colonisation success. This theory received empirical support in a recent study of seed beetles (*Callosobruchus*
50 *maculatus*), in which populations founded by polyandrous females had increased fitness after five generations
51 compared to monogamous females [18]. Interestingly, despite the benefits of polyandry for individual fitness,
52 Power and Holman [18] found no effect of mating treatment on extinction rates, which were low throughout
53 the experiment. It is therefore not yet known how much of a benefit polyandry may provide in terms of

establishment probability in harsh environments. Given that inbreeding depression is most severe in poor environmental conditions [19], this is an important area for future investigation.

The red flour beetle *Tribolium castaneum* is an ideal model to test how polyandry influences colonisation success in a new environment. A pest of stored products, the ecology of *T. castaneum* is characterised by colonisation of empty habitats (e.g grain stores), presumably often by a small number of founders [20]. Experimental studies in this species have shown that founder effects have pronounced costs as a result of genetic and demographic effects, and that colonising populations are able to rapidly adapt to novel environments [21,22]. Further, this species is highly promiscuous and experimental evolution studies have shown that a history of sexual selection results in decreased risk of extinction under inbreeding, and in improved invasion of competitor populations [23,24]. A substantial proportion of matings in this species fail to result in offspring being produced, and there is some evidence to suggest that these costs are reduced when females mate multiply [25]. However, we do not yet know how polyandry affects colonisation success in a novel environment.

The aim of this study is to test how polyandry affects colonisation dynamics in a novel thermal environment. We placed singly and doubly mated *T. castaneum* females into an empty habitat at high temperature, and allowed populations to grow for ten generations. We first tested the hypotheses that, i) populations founded from polyandrous females were less likely to go extinct, and ii) polyandrous populations exhibited larger population sizes due to increased reproductive fitness. We use these findings to determine how mating strategy and inbreeding interact to affect colonisation dynamics in novel thermal environments.

Materials and Methods

Experimental protocols

All beetles were of our Krakow Super-strain (KSS) [26] and were maintained both before and throughout the experiment on a fodder medium consisting of 90% organic strong white bread flour mixed with 10% brewer's yeast, and topped with a thin layer of oats for traction.

Founding females and their mates were reared separately and mated under standard conditions of 30°C and 60% humidity. Matings were carried out in 7ml screw-top vials containing 1.5g of fodder. All females received two matings, each lasting 24 hours. In the first round of matings, random pairs of virgin females and virgin males (aged ~7 days post-eclosion) were combined. In the second round of matings, half of the females from the polyandrous treatment were mated to a second, non-virgin male, hereafter referred to as the 'polyandrous' treatment. The remaining females were assigned to a 'monogamous' treatment, in which

they were re-mated to the same male, who, for consistency between treatments, was briefly removed from the dish before being replaced.

Using the above mating treatments, we established populations in a novel thermal environment for the remainder of the experiment. Specifically, after 48 hours of mating with either one or two males, females were transferred to a population container (100 ml PVC screw-cap containers, with the caps pierced for ventilation, containing 70 ml fodder) and left to oviposit for 7 days at 38°C and 60% humidity, after which they were removed and the offspring left to develop. This temperature is at the upper limit at which *T. castaneum* can reproduce, and constitutes a highly stressful environment [27]. All population containers post-mating were marked only with a randomised ID number so that experimental treatment was unknown by researchers during subsequent handling. Thirty-five days after females were removed, the first generation of offspring were separated from the fodder by sieving, the fodder was discarded and the container and sieve cleaned with ethanol between replicates. The number of live adults was counted and placed into fresh fodder to seed the next generation. If >100 individuals were present, 100 were retained to mate in the next generation and the remainder discarded after counting (in order to minimise density-dependent effects). This new generation of adults was allowed to mate and lay on the fresh fodder for seven days, after which adults were removed by sieving and the offspring again left to develop for 28 days. This process was repeated for 10 generations, all at 38°C.

Statistical analyses

All analyses were carried out using R version 3.3.3 [28]. We separately modelled how the experimental mating treatment affected i) the probability of extinction over ten generations, and ii) changes in population size over the same period. For the extinction analysis, we used Cox proportional hazards models, implemented in the Survival package [29] in R. Because some populations went extinct in the first generation, possibly as a result of failure to mate and/or conceive, we ran the survival models both with and without populations that went extinct in the first generation.

To model how population size changed over time, we used generalised linear mixed models, implemented using the glmmADMB package [30] in R. For this analysis we only included population counts above zero. Offspring number per generation was modeled as a response variable with a negative binomial error distribution, and generation and experimental treatment (monogamous vs polyandrous founder) were fitted as explanatory variables. To account for potential non-linear changes in population size over time, we constructed three models, with generation fitted as i) a continuous variable, ii) a fixed factor and iii) a third order polynomial. For all models, population ID was fitted as a random effect. Finally, we tested for a difference in population

size between experimental treatments in the first and last generation, using two separate generalized linear models (as above but with no random effects), implemented using the MASS package [31] in R.

Results

We tracked a total of 53 monogamous and 55 polyandrous *T. castaneum* populations in a novel thermal environmental until extinction, or for 10 generations. Overall dynamics of the populations are given in Figure 1; though there was a general trend for increasing population size, there were substantial fluctuations over generations, with decreases in population size between generations three and four, and between generations six and seven (Fig. 1). Despite this, we observed a clear and consistent trend of larger adult population size in populations founded by polyandrous compared to monogamous females (Fig. 1).

For statistical comparison of our experimental treatments, we separately tested for differences in extinction rates (i.e. did time to extinction differ between treatments), and population size changes over time. In the first generation, six populations founded by monogamous females went extinct (11%), while no populations founded by polyandrous females went extinct. By generation ten, 18 monogamous populations (34%) and five polyandrous populations (9%) were extinct (Fig. 2A). The effect of treatment on time to extinction was significant (Cox proportional hazards; hazard ratio = 0.256; 95% CIs = 0.102, 0.642; $P = 0.004$). This effect remained significant after removal of populations that went extinct in the first generation (hazard ratio = 0.361; 95% CIs = 0.137, 0.950; $P = 0.039$).

We next tested how founder mating regime affected fitness and population trajectories. Excluding extinctions, we found no significant difference in the number of offspring produced by monogamous or polyandrous females in the first generation (GLM, $P = 0.503$). Considering all generations, however, we found that populations founded from polyandrous females had higher overall population sizes than populations founded by monogamous females (Fig. 2B, Table 1). We also found that, when generation modeled as a continuous variable, population size increased over time, but that there was no interaction between treatment and generation (Fig. 2B, Table 1). The effect of experimental treatment was also significant when generation was modeled as a fixed factor ($P = 0.037$), and as a third-order polynomial ($P = 0.018$). Finally, considering only populations that survived all ten generations, population size in polyandrous-founded populations in generation ten was significantly higher in monogamous-founded populations (GLM, $P = 0.004$).

Discussion

The costs of mating with multiple males are likely to outweigh any direct benefits gained, and as such the evolution and maintenance of polyandry are generally viewed as an evolutionary puzzle [32]. Potential indirect benefits of polyandry include i) enabling sexual selection, ii) enabling bet-hedging, and iii) reducing inbreeding load. Previous research using the *T. castaneum* model has found that long-term sexual selection results in increased population fitness [23,24]. Here we show that polyandry has substantial benefits in colonising populations, even when the opportunity for sexual selection is reduced.

Perhaps the best-studied way in which females can increase the fitness of their offspring through polyandry is through bet-hedging [16]. By mating with multiple males, females may reduce the risk of being fertilised by an unsuitable male, and therefore increase offspring fitness. Bet-hedging is likely to be most beneficial when i) there is a substantial proportion of highly unsuitable (e.g. infertile) males in the population, and ii) the population is small [16]. In our study, populations were founded by a single female, and as such there is clear potential for polyandry to provide benefits. We found that 11% of monogamous females produced no offspring in the first generation, while all polyandrous females produced offspring. This is consistent with a situation in which a failure to produce offspring is the result of male infertility, under which we would only have expected 1.28% of random pairs of males to both be infertile. The mating conditions we imposed were short and artificial, and studies of natural populations are required. Nevertheless, previous research in *T. castaneum* has found that a substantial proportion of matings fail to result in offspring production [25], and across insects, high levels of male infertility have been observed in the wild [33]. It is therefore likely that multiple mating is an important mechanism for increasing short-term establishment probability in newly-colonised populations.

A second mechanism through which polyandry can benefit colonising populations in the absence of sexual selection is through reducing levels of inbreeding in subsequent generations [17]. Consistent with this hypothesis, we found significantly lower population sizes and higher extinction rates in monogamous-founded populations over the full duration of our experiment. Population sizes fluctuated substantially over the course of our experiment, likely a result of density-dependent processes which are well-documented in *T. castaneum* [34]. Though the higher population sizes in polyandrous-founded populations were generally consistent over time, it is notable that the difference between treatments was highest when population sizes were high (i.e. in generations 2,3,8 and 9), and lowest when population sizes were reduced (i.e. generations 4 and 7). It is possible that a bet-hedging like scenario could explain these longer-term benefits of polyandry if there was substantial variation in fitness among fertile males, as multiple-mating would increase the chances

of mating with at least one suitable male [16]. However, this is unlikely to explain our results, as we found no difference in population size between treatments in the first generation, but instead only when considering later generations. Similarly, if post-copulatory sexual selection could explain some of the differences observed between our experimental treatments, we would expect to observe at least some differences in offspring fitness in the first generation. We therefore suggest that in *T. castaneum* and similar systems, polyandry benefits colonising populations through i) guarding against male infertility and enabling establishment, and ii) reducing inbreeding and enabling longer-term population persistence.

Our results are broadly consistent with a recent study in *C. maculatus*, in which the increased fitness in polyandrous-founded populations were observed in F4 and F5, but not F1-F3, generations [18]. It is worth noting that, unlike in our study, Power and Holman [18] found no effect of mating treatment on extinction, likely because of the environment was relatively benign, or because there was insufficient time for extinctions to occur. Here, through a longer-term experiment, we demonstrate that the benefits of polyandry can persist for substantial periods of time, and show that they are likely to be important for populations colonising novel environments.

Finally, it is worth noting that our study does not aim to address whether the benefits gained from either bet-hedging or inbreeding avoidance are sufficient to explain the evolution of polyandry [11,17]. Rather, we suggest that in the specific context of colonising novel environments, the benefits of these processes are likely to be substantial [16]. Future climate change is expected to result in species shifting their ranges and undergoing changes in population size, and there is increasing realisation that evolutionary processes need to be incorporated into predictive models of population and species responses to climate change [35]. The multiple, interacting benefits of polyandry should certainly be incorporated into such models in order to improve predictive power.

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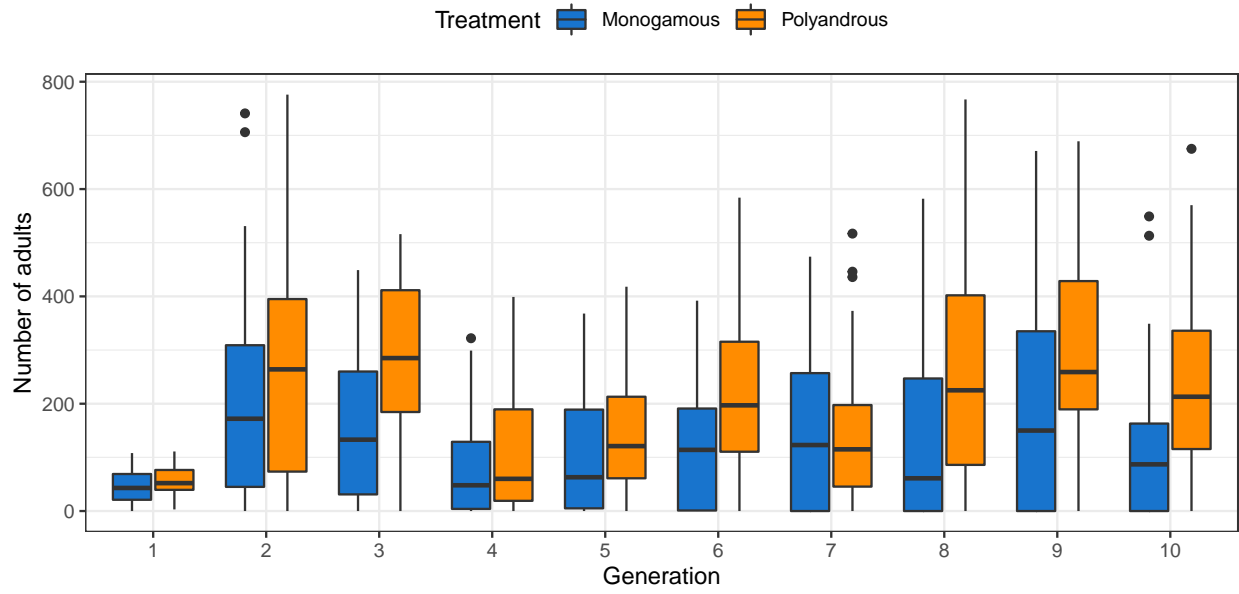
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Tables and Figures

Table 1 Summary of results from a generalised linear mixed model of population dynamics of experimental *T. castaneum* populations founded from monogamous or polyandrous females. Here, the ‘treatment’ estimate refers to the effect of polyandrous relative to monogamous females, and generation was modeled as a linear effect. Population ID was modeled as a random effect (Var = 0.12, SD = 0.35).

	Estimate	SE	P
(Intercept)	4.716	0.084	< 0.001
Treatment	0.227	0.090	0.012
Generation	0.065	0.010	< 0.001
Treatment x Generation	0.005	0.020	0.815



277

278 **Figure 1** Colonisation dynamics of experimental *T. castaneum* populations founded from monogamous or
 279 polyandrous females.

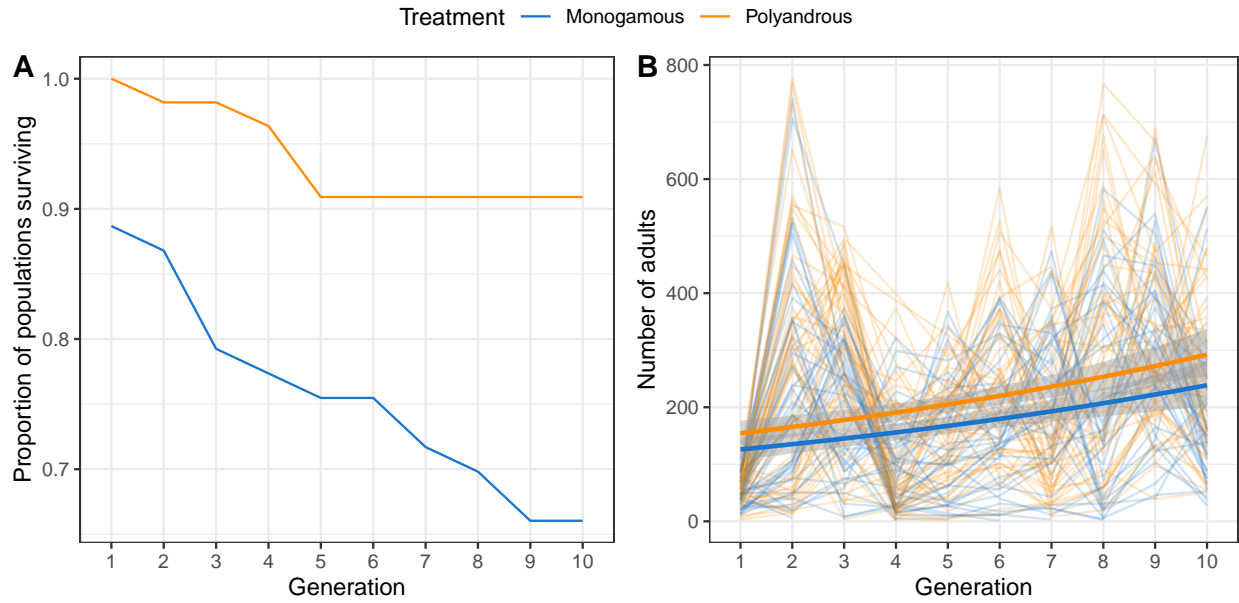


Figure 2 Extinction and population dynamics of experimental *T. castaneum* populations founded from monogamous or polyandrous females. **A** Proportion of populations surviving over time; **B** number of adults in experimental populations. In **B**, thin lines represent individual populations, while the thick lines represent fitted values from a negative-binomial GLM.