

## Inbreeding decreases promiscuity in *Drosophila melanogaster* females

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*Received 31 August 2014, accepted 14 March 2015*

Polyandry and promiscuity by females are widespread phenomena in natural populations despite the costs associated with multiple matings. Previous studies using flour beetles (*Tribolium castaneum*) and field crickets (*Gryllus bimaculatus*) suggest that these behaviors are more common in inbred individuals, perhaps as a mechanism to ensure genetic diversity in the offspring produced or due to reduced choosiness. However, using *Drosophila melanogaster*, we observed the converse pattern; relatively inbred females were less promiscuous than relatively outbred ones both in terms of the number of males and the number of phenotypes of males chosen, and took longer to copulate which is consistent with increased choosiness. The effects of inbreeding on behavior may therefore be different across different species and may depend on tradeoffs that differ between species rather than follow a general rule.

KEY WORDS: *Drosophila*, female mate choice, inbreeding, promiscuity, polyandry.

### INTRODUCTION

In his classic paper, BATEMAN (1948) concluded that males should be the indiscriminate sex while females should be choosy and restrict their mating to the best available male. However, this does not seem to be universally true (BONDURIANSKY 2001; GOWATY et al. 2003; BYRNE & RICE 2006; EDWARD & CHAPMAN 2011). Bateman's own study organism, *Drosophila melanogaster*, demonstrates polyandrous behavior (MARKS et al. 1988), which presents a paradox because of the negative effects of mating events (PITNICK & GARCIA-GONZALEZ 2002). The negative effects that have been found in females who mate multiple times include decreased life span (FRIBERG & ARNOVIST 2003) and fitness (WIGBY & CHAPMAN 2005). Since one male can provide sufficient sperm to inseminate a female for several weeks (LEFEVRE & JONSSON 1962), if females can identify the best male, then selection to avoid the negative effects of polyandry would be expected to have occurred and led to an absence of polyandry. Indeed, polyandry alone was not found to have direct fitness benefits in *Drosophila melanogaster* (BROWN et al.

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2004), which begs the question of the continued existence of this phenomenon. However, if the presence of polyandry in this species is able to rescue the fitness of some types of female *Drosophila*, specifically ones of lower or higher quality, selection within these subsets of females may explain the presence this behavior.

One potential factor that may provide selection for polyandry is inbreeding. Inbreeding causes a wide range of impacts on individuals and populations; most predominately it causes inbreeding depression, which is defined as a lower mean fitness for a population due to the decreased heterozygosity arising from inbreeding. This reduced heterozygosity causes an increased expression of recessive deleterious genes that would normally be hidden in heterozygotes (CHARLESWORTH & CHARLESWORTH 1987). Inbreeding has been shown to cause lower egg-adult viability (EHIOBU et al. 1989; ROBINSON et al. 2009), lower fecundity (EHIOBU et al. 1989; KRISTENSEN et al. 2011) and shortened life span (VALTONEN et al. 2011) in *D. melanogaster*. Along with these life-history traits, there is evidence that the ability of *Drosophila* to learn is also negatively affected by inbreeding (NEPOUX et al. 2010). Learning has been shown to affect mate choice in female fruit flies (DUKAS 2005) and allow greater reproductive success due to increased courtship of conspecifics over heterospecifics (DUKAS 2008; DELBARCO-TRILLO et al. 2010). Reductions in learning ability in inbred females may therefore act to select in favor of females that compensate for this during states of low heterozygosity in some manner.

With no evidence for pre- or post-copulatory avoidance of inbreeding in *Drosophila* (ALA-HONKOLA et al. 2011; TAN et al. 2012), inbreeding may be a widespread issue in natural populations. Mechanisms that reduce the negative effects of inbreeding by increasing the genetic variation of the offspring may therefore be selected for. MICHALCZYK et al. (2011) found that inbred female flour beetles, *Tribolium castaneum*, were more promiscuous than their outbred conspecifics and that the more promiscuous inbred females were more fecund than the monogamous inbred females. Similarly, field crickets, *Gryllus bimaculatus*, appear to receive a fitness-rescuing effect through polyandry in inbred females (TREGENZA & WEDELL 2002).

We acknowledge that our experiment varies from the previous experiments in the degree of polyandry expressed in the model organism as well as in the design. These other experiments served as a motivation for a possible explanation of the presence of polyandry found in *Drosophila melanogaster* females that has been described many times (e.g., MARKS et al. 1988; BROWN et al. 2004; BYRNE & RICE 2005). However, many of the *Drosophila* experiments failed to find an explanation for the continued presence of this behavior. We therefore conducted this study to test a possible explanation for the presence of inbreeding in these populations, and not as an attempt at directly replicating either the TREGENZA & WEDELL (2002) experiment or the MICHALCZYK et al. (2011) experiment.

We therefore expect that a similar fitness-rescuing technique could be selected for in *D. melanogaster*, and predict that inbred females should be more promiscuous than their otherwise similar outbred counterparts. Additionally, we expect that those inbred females who are more promiscuous and indiscriminate will have a higher fecundity than the monogamous inbred females. To test these predictions, we performed an experiment using two parallel lines of *D. melanogaster* that differed in their degree of inbreeding, and recorded their mate choice behaviors and fecundities.

## MATERIALS AND METHODS

*Fly husbandry*

Females used in the experiment were derived from a *D. melanogaster* wild-type population obtained from Dr Michael Rose; these lines are descended from 200 males and 200 females collected by P.T. Ives in South Amherst, Massachusetts in 1975 (ROSE & CHARLESWORTH 1981a, 1981b). These genetically variable lines have been used in a number of other studies, but mainly for physiological evolution (e.g., KIMBER & CHIPPINDALE 2013; MUELLER et al. 2013). Stock populations of *D. melanogaster ebony* and *yellow* mutants used to generate the males for the experiment were derived from two mutant lines supplied by the Bloomington Stock Center, Bloomington, Indiana (Lines numbers 1658 and 169 respectively). All flies were maintained in laboratory conditions under a 12:12 hr light:dark cycle at 25 °C in shell vials (95 mm height, 30 mm diameter) with five females and five males per vial. Flies were fed with a standard corn meal-based food mixture.

*Fly inbreeding*

The inbred line was established by performing eight generations of full sibling-to-sibling matings. The resulting inbreeding coefficient, assuming no prior inbreeding effects, would therefore be  $F = 0.7852$ . After the eight generations of inbreeding, the lines were then maintained by performing random  $5 \times 5$  matings in a similar manner as was done to the parallel outbred female lines.

*Mate choice*

Mate choice was performed using virgin inbred wild type females, virgin outbred wild type females, and virgin *yellow*, *ebony* and wild-type males. The experimental vials contained standard food and were divided by a thin piece of plastic. One virgin female was placed on one side of the divider and one virgin male of each phenotype was placed on the other side, for a total of three males. These vials were labeled with a number unique to the female and whether she was from the inbred or outbred line. These vials were all then placed in an incubator for 24 hr to allow the flies time to acclimate.

After 24 hr, the dividers were removed and the flies were watched continuously for 8 hr, and all copulations and phenotypes of the males copulated with were recorded along with the respective times in minutes. A 24-hr acclimation period prior to assessment of mate choice using these devices has been used previously (MCKEE et al. 2014).

*Fecundity*

After 8 hr of observations, the males were removed and the females remained in the vials. After 2 weeks, the females were removed and the eclosing offspring were counted every morning for an additional 7 days. Labels on the vials allowed us to associate the total number of copulations and number of male phenotypes copulated with to the number of subsequent offspring.

## Data analysis

The data were analyzed using a combination of analyses of variance (ANOVAs) and Student's *t*-tests for the fecundity analysis and mate choices. A Mann-Whitney test was used to analyze the copulation latency data. These were performed using the Mini-tab program (v. 16.2.4).

## RESULTS

## Mate choice

Fig. 1 shows the results of the mate choice experimental trials. Inbred females mated significantly fewer times than outbred females (*t*-test,  $t_{271} = 3.00$ ,  $P = 0.003$ ), and mated with a lower number of different phenotypes (*t*-test,  $t_{262} = 4.77$ ,  $P = 2.77 \times 10^{-6}$ ). Inbred females also had significantly longer copulation latency than outbred females (Mann-Whitney test,  $W = 16175.5$ ,  $N_1 = 147$ ,  $N_2 = 148$ ,  $P < 0.001$ ).

## Fecundity

Fig. 2 shows the results of the fecundity measurements. Inbred females produced significantly fewer offspring than outbred females (*t*-test,  $t_{242} = 7.61$ ,  $P = 1.42 \times 10^{-12}$ ). Overall, with the combination of both groups of females, there was a significant drop in fecundity when individuals mated 3 or more times when both treatments were combined (one-way ANOVA,  $F_2 = 3.55$ ,  $P = 0.030$ ), but this was not significant when analyzed separately for inbred and outbred females (one-way ANOVA,  $F_2 = 1.28$ ,  $P = 0.283$  and  $F_2 = 2.22$ ,  $P = 0.112$ , respectively). Females who mated twice showed no significant increase in fecundity compared to inbred females who only mated once (unpaired

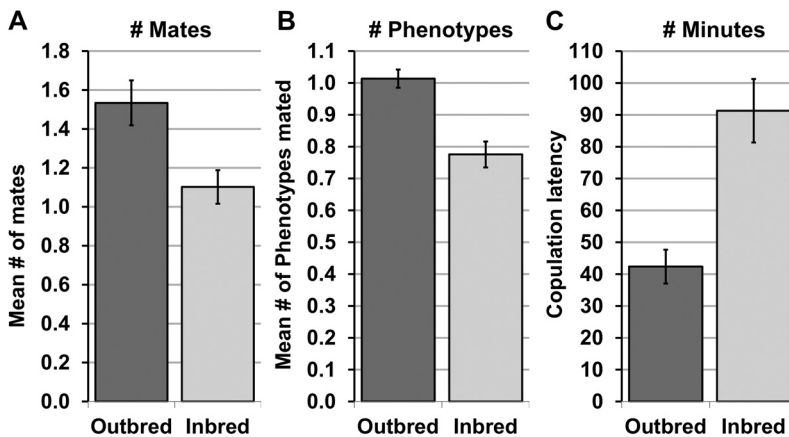


Fig. 1. — Results of mate choice experiment. (A) Outbred females mated significantly more often than inbred females (heteroscedastic *t*-test,  $t_{271} = 3.00$ ,  $P = 0.003$ ). (B) Outbred females mated with significantly more distinct male phenotypes than inbred females (heteroscedastic *t*-test,  $t_{262} = 4.77$ ,  $P \ll 0.001$ ). (C) Outbred females had a significantly shorter copulation latency than inbred females (Mann-Whitney test,  $W = 16175.5$ ,  $N_1 = 147$ ,  $N_2 = 148$ ,  $P < 0.001$ ). Bars indicate standard errors.

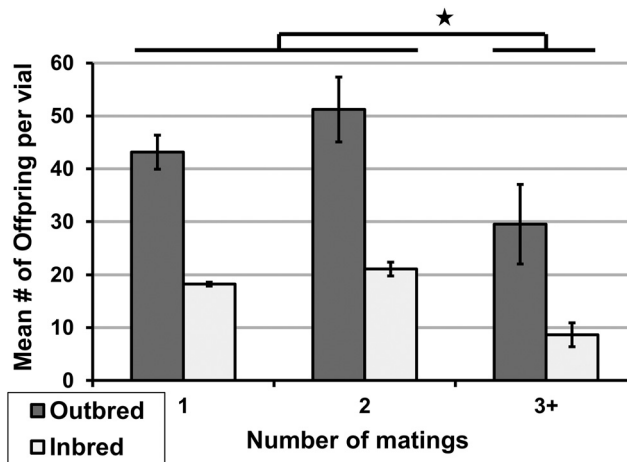


Fig. 2. — Fecundity measurements for mated females. The average number of offspring produced was significantly higher in outbred females than inbred females in all three categories (heteroscedastic t-test,  $t_{242} = 7.61$ ,  $P \ll 0.001$ ). Additionally, the overall average number of offspring produced was significantly higher for females who mated with one or two males compared to those who mated with three or more (one-way analysis of variance (ANOVA),  $F_2 = 3.55$ ,  $P = 0.030$ , indicated by the star symbol). This equated to a 42% decrease in outbred female fecundity and a 59% decrease of fecundity in inbred female fecundity for those who mated 3 or more times compared to 2 times. No other differences between categories were statistically significant. Bars indicate standard errors.

heteroscedastic t-tests; overall:  $t_{29} = 0.956$ ,  $P = 0.056$ , inbred:  $t_{29} = 0.769$ ,  $P = 0.297$ , outbred:  $t_{29} = 0.915$ ,  $P = 0.107$ ).

## DISCUSSION

Overall, the data did not match the initial predictions of increased promiscuity in inbred females, and the converse was actually observed. While our experiment was motivated by the behaviors reported for *Tribolium castaneum* (MICHALCZYK et al. 2011) and *Gryllus bimaculatus* (TREGENZA & WEDELL 2002), we acknowledge major differences in our experimental design compared to these other experiments (and are therefore not trying to directly compare our results to the those studies), but the overall results are still in contrast to those we would expect based on those previous studies.

Relatively inbred females not only mated with fewer male phenotypes and had a lower number of total matings, but they also had a significantly longer latency period before copulation (see Fig. 1). Time to copulation and copulation successes are frequently used in mate choice experiments as proxies for mate preferences (e.g., FRIBERG & ARNOVIST 2003; ÖDEEN & MORAY 2008; TAN et al. 2013) so our data therefore seem to indicate that the inbred females were more selective instead of more indiscriminate relative to the outbred females. The totality of our results therefore suggests that, in *D. melanogaster*, inbreeding may actually increase female choosiness while decreasing promiscuity.

Overall, all the inbred females had a significant decrease in fecundity compared to outbred females, as would be expected from multiple generations of sibling-sibling. We

are confident that this reduction in fecundity is due to the inbreeding because our flies were raised in nearly identical conditions, which would minimize the possibility for other factors to cause variation in fecundity. Additionally, since we saw a consistently lower fecundity in inbred females compared to outbred females regardless of the number of mates, this provides further evidence that the lower fecundity is based on the inbreeding of the females and not due to differences in male mate choice preferences.

Being more selective may be advantageous for the inbred females because of the decrease in their fecundity when inbred females mated three or more times (see Fig. 2). Females who mated twice, in both treatments, had non-significantly more offspring on average than females who mated once, but females in both of these categories had significantly more offspring than females who mated three or more times. This finding may support the findings for an optimal intermediate level of polyandry in females (ARNOVIST & NILSSON 2000). There was a dramatic overall effect of being inbred on female fecundity; in the case of the inbred females, those who mated twice still did not have as many offspring as the least fecund group (three or more matings) of the outbred females. This drastic reduction in offspring output may accentuate selection within inbred females to mate fewer times in order to maximize their relative offspring output (e.g., inbred females who mated 3 or more times had on average 41% the number of offspring of those who mated twice, while outbred females who mated 3 or more times had 58% the number of offspring of those who mated twice).

There is evidence that polyandry may be beneficial for females in various species. Some examples of this include increased offspring heterozygosity in blue tits (FOERSTER et al. 2003), evidence of the same in *Drosophila* (IMHOF et al. 1998), increased offspring viability in *Drosophila* (GOWATY et al. 2010) and increase in offspring production in *Drosophila* (TAYLOR et al. 2008), and multiple paternities would allow more genetic variation within offspring clutches in *Drosophila melanogaster* (OCHANDO et al. 1996). However, multiple matings have a wide range of costs (FRIBERG & ARNOVIST 2003; WIGBY & CHAPMAN 2005) which inbred females may be less able to endure. Our results suggest that the balance between the benefits and costs of polyandry results in a higher degree of polyandry for outbred than for inbred females in *D. melanogaster*.

There is also evidence of indirect benefits of polyandry in *Drosophila melanogaster*, where the mother suffers the costs, but benefits are passed onto the daughters (PRIEST et al. 2008). If this is the case, it would further support the explanation that the increased polyandry found in outbred females is beneficial, while the costs are too great for the less-fit inbred females.

If these differences in behavior are driven by such a balance, then changes in promiscuity and choosiness may rely on the existence of some mechanism by which females are able self-assess their own physiological state or quality. Females of poor quality, due to inbreeding, or perhaps even some other factor, may have mechanisms to detect this fact and adjust their mating behaviors in an appropriate manner. This self-assessment ability has been reported for some species (HEBETS & SULLIVAN-BECKERS 2010; SOPER et al. 2014). In this case, inbred females may be behaving in the manner that is most optimal for fecundity.

Alternately, the loss of learning ability occasionally found due to inbreeding (NEPOUX et al. 2010) may also account for the difference in choice behaviors we observed. If females rely heavily on their previous experience for mate choices (BILLETER & LEVINE 2012) or use some other complex cognitive selection procedure, then there may be problems in the mate choice abilities of the inbred females. These

problems would explain the copulation latency we observed in the inbred females as arising not from increased choosiness, but rather from poorer decision-making abilities.

Future directions to further clarify these results may include a larger experiment that includes multiple independent inbred lines that could help establish this as a species-wide fixed behavioral plasticity, and not just an artifact of one inbred line that has this property. Also, a design to look at how polyandry varies with the presentation of related and unrelated males, as in TREGENZA & WEDELL (2002), could be quite interesting. In this scenario, we would predict similar results because *Drosophila* do not appear to have inbreeding avoidance (ALA-HONKOLA et al. 2011; TAN et al. 2012).

There is a need for more research to help detangle these possible explanations for our observations and why there are apparently contrasting observations seen in other taxa, other than differences in experimental design (TREGENZA & WEDELL 2002; MICHALCZYK et al. 2011). At the least, our results show it is important to consider that the effects of inbreeding on behavior may be manifested in different ways in different systems, and reports from single species (e.g., BATEMAN 1948) should not be taken as a universal rule or process.

#### ACKNOWLEDGEMENTS

We would like to thank all of the undergraduate assistants who helped throughout this process: Grace Lee, Annessa Du, Jose Felix Cruz, Sam Degregori, Brent DeShazo, Lilhac Medina, Breanne Fletcher, Marissa Andaloro, Phuong Hyun, Amisha Thakkar and Natalie Lozano.

A special thanks to Louis Stokes Alliance for Minority Participation (LSAMP), which was vital for funding this project (grant no. HRD-0802628).

#### DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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