

# Influence of Inbreeding on Female Mate Choice in Two Species of *Drosophila*

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**Abstract** Many organisms have been reported to choose their mates in order to increase the heterozygosity of their offspring by avoiding mating with relatives or homozygous individuals. Most previous studies using *Drosophila melanogaster* have used artificial chromosomes or extreme inbreeding treatments, situations unlikely to be matched in nature. Additionally, few studies have examined the interaction between female inbreeding status and her choice of mate. Using females and males from populations that had experienced either random mating or one generation of sib-sib inbreeding, we measured the preferences of females for males. Our results indicate that outbred males were chosen more often than inbred males and that this preference may be more pronounced in outbred females than in inbred ones.

**Keywords** Inbreeding · mate choice · *Drosophila* · homozygosity · heterozygosity

## Introduction

Mate choice behaviors play an important role in the evolution of many species (Darwin 1871; Andersson 1994). Mate choice has been found to occur in organisms as diverse as marine crustaceans (Palmer and Edmands 2000), reptiles (Laloi et al. 2011) and mammals (Hoffman et al. 2007; Ilmonen et al. 2009). A number of secondary sexual characteristics appear to have evolved via this sexual selection and a number of models have been proposed to describe the many facets involved (Andersson 1994; Palmer and Edmands 2000; Bonneaud et al. 2006; Kempenaers 2007; Sherman et al. 2008; Ilmonen et al. 2009; Griggio et al. 2011). Most often, the size or extravagance of secondary sexual traits are taken to be an indicator of general health and are most influential for mate choice, but more subtle aspects of potential partners associated with

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their genetic homozygosity or relatedness may themselves be under selection (Ilmonen et al. 2009; Laloi et al. 2011; Pölkki et al. 2012).

The negative effects of inbreeding (inbreeding depression) have long been known (Darwin 1876). Too much inbreeding can even result in a decrease in the size of a population, as a result of less fit individuals being born, potentially posing a risk to the population's persistence (Miller et al. 1993). The negative effects of inbreeding are really those of low heterozygosity (Charlesworth and Charlesworth 1987; Lynch and Walsh 1998), and any mechanism that avoids low heterozygosity in offspring would have similar benefits as inbreeding avoidance. Along these lines, Brown (1996) suggested that females would choose mates which would increase the heterozygosity of their resulting offspring. A study on blue tits (*Cyanistes caeruleus*) showed evidence for female sexual selection on a trait influenced by heterozygosity; more heterozygous males provided more parental care and offspring from social pairs with high heterozygosity had higher degrees of heterozygosity themselves (García-Navas et al. 2009). In wire-tailed manakin (*Pipra filicauda*), females select more heterozygous males who tend to have larger wing and tarsus sizes as well as better success in acquiring territories (Ryder et al. 2009).

Inbreeding is not completely deleterious however; by choosing close relatives, females may be ensuring genetic compatibility (Bonneau et al. 2006) or favoring their own alleles via a form of kin selection (Kokko and Ots 2006). Additionally, by exposing deleterious alleles to selection, inbreeding can foster the eradication of recessive deleterious alleles from a population and thereby reduce long-term genetic load (Quilichini et al. 2001).

The opposite of inbreeding is outbreeding, biased mating with non-relatives. The advantage of outbreeding has long been recognized due to the widespread phenomenon of hybrid vigor, when members of the F1 generation of a cross between two genetic lines have higher fitness (Marques et al. 2011; Seko et al. 2012; Shahid et al. 2012). Outbreeding is not always advantageous however, choosing distantly related individuals can cause reductions in fitness in the F2 and succeeding generations (outbreeding depression) due to the breakdown of co-adapted gene complexes (Quilichini et al. 2001; Vorsino et al. 2012).

Although arguments can be made for the benefits and detriments of both inbreeding and outbreeding, the consensus is that inbreeding poses a larger threat to the fitness of a particular organism or population than outbreeding (Miller et al. 1993; Palmer and Edmands 2000; Ilmonen et al. 2009; Välimäki et al. 2011). For this reason, we expect the overall net effect of an evolutionary history of selection on mate preferences to result in adaptations to reduce the risk of low heterozygosity in offspring; either by avoiding inbreeding itself or by assessing the genetic homozygosity or similarity of potential mates and choosing more heterozygous partners.

Previous studies have shown that inbreeding has an effect on mate choice behaviors in several organisms with avoidance of relatives and likely relatives demonstrated in a number of species (Kempnaers 2007). In copepods (*Tigriopus californicus*), females chose random males significantly more often than male siblings (Palmer and Edmands 2000) and in fruit flies (*D. montana*), females required a longer courtship time from more closely related males than from males from another strain (Suvanto et al. 2000). The processes that reduce inbreeding can be subtle; female semelparous treehoppers (*Umberonia crassicornis*) do not distinguish between related and unrelated individuals of

the same age, but their preference for older individuals causes an overall avoidance of inbreeding (De Luca and Cocroft 2008).

There is also evidence for mate selection to avoid outbreeding however. A study on Peron's tree frog (*Litoria peronii*) showed that the sperm of males who were more closely related to the female fertilized more of her eggs than did the sperm of more distantly related males, possibly due to cryptic female choice (Sherman et al. 2008). In the wire-tailed manakin (*Pipra filicauda*) study cited above (Ryder et al. 2009) the females preferentially mated with related males more than expected under random mating. Thünken et al. (2011) report that selective cichlids (*Pelvicachromis taeniatus*) in their study preferred relatives to unrelated individuals and in another study, treehopper females (*Umbohia ataliba*) preferentially mated with their brothers (Masters et al. 1994). Finally, an experiment with house sparrows (*Passer domesticus*) indicated that males with either low MHC diversity or MHC alleles that were too dissimilar from those of the females were not chosen for copulation (Bonneauud et al. 2006).

Avoidance of inbred individuals, regardless of their relatedness, has also been demonstrated in several species. Female mice (*Mus musculus musculus*) prefer the scent of outbred males to inbred males when both were infected with salmonella (Ilmonen et al. 2009) and female mealworm beetles (*Tenebrio molitor*) prefer the scent of outbred males to inbred males (Pölkki et al. 2012), but Aparicio et al. (2001) found that males of spotless starling (*Sturnus unicolor*) with an intermediate level of heterozygosity were more successful in mating and reproduction than either of the extremes. These preferences may arise from reduced fitness in females who mate with inbred males instead of outbred ones as demonstrated in seed-feeding beetles (Fox et al. 2012).

Additionally, the degree of homozygosity of the female herself may influence the degree to which male homozygosity is weighted in mate choice behaviors, but observed patterns of preference seem mixed (Kempenaers 2007). Examples include studies showing that blue tit (*Cyanistes caeruleus*) females preferred more heterozygous males with a correlation between heterozygosity in social pairs indicative of stronger preference in heterozygous females than homozygous ones (García-Navas et al. 2009), lower choosiness being exhibited by inbred zebra finch females (*Taeniopygia guttata*) (Bolund et al. 2010) and less pronounced preferences for heterozygous males by females with lower MHC heterozygosity in sticklebacks (*Gasterosteus aculeatus*) (Reusch et al. 2001). In contrast, female sparrows (*Passer domesticus*) with lower MHC heterozygosity preferred males with higher MHC heterozygosity whereas more heterozygous females did not show a preference (Griggio et al. 2011) and inbred three-spined stickleback (*Gasterosteus aculeatus*) females showed stronger preferences for artificial males than outbred females (Mazzi et al. 2004). Finally, the inbreeding status of the female did not appear to influence mate choices between siblings and unrelated males in guppies (*Poecilia reticulata*) (Guevara-Fiore et al. 2010) or sticklebacks (*Gasterosteus aculeatus*) (Frommen and Bakker 2006).

These previous studies suggest that inbreeding avoidance and outbreeding avoidance may be working simultaneously in organisms and may be contingent on the heterozygosity of the choosing individual. The combination of both of these factors may account for the lack of preference for or against relatives seen in some studies (e.g., Viken et al. 2006; Tan et al. 2012). To better understand the degree to which females may use the homozygosity of potential mates and their own degree of

homozygosity to influence their mating preferences we examined the effects of male and female inbreeding status on female mate choice in *Drosophila*. We examined three lines from two species of *Drosophila* to allow broader conclusions than a study conducted purely within one line in a single species.

Using genetically variable initial populations, two generations of crosses were used to generate pairs of relatively inbred and outbred males who were then presented to relatively inbred and outbred females for mating. The females' preferences were then recorded and related to the genetic status of the males and females and the observations compared to several predictions. First, the inbred treatments should have increased homozygosity and therefore reduced genetic quality which leads us to predict that females will prefer outbred males over inbred ones. Second, this reduced genetic quality of the inbred females themselves may influence the strength of their mate choice preferences. If increased homozygosity leads to stronger female preference then we would expect to see the inbred females exhibit a stronger preference for outbred males than outbred females do. If increased homozygosity leads to less choosiness then we would expect to see the inbred females exhibit a weaker preference for outbred males than outbred females do. Finally, we expect the time taken for females to choose a mate to be less when the females have a stronger preference as is typical for studies with *Drosophila* (e.g., Dukas 2005; Taylor et al. 2007).

## Methods and Materials

### Fly Maintenance

Fly lines were started from three initial genetically variable lines and mated only within their own line. The initial lines were called Irene (*D. simulans*), Jon (*D. simulans*), and Rose (*D. melanogaster*). The Irene and Jon lines were collected from two locations in Los Angeles County (Compton and Inglewood) in June of 2011 approximately 3 months prior to the experiment and are the result of mass matings from an initial 80 and 23 isofemale lines, respectively. The Rose line was obtained from Michael Rose at the University of Irvine two months prior to the experiment. This line is quite genetically variable (e.g., Mueller et al. 2013). The recent initiation of these lines, large maintained population size and trend toward reduced size in the inbred treatments compared to the outbred ones (see results) indicate segregating genetic variation present in these lines.

Flies were maintained in an incubator at 25°C with cycles of 12 h of light and 12 h of dark. They were kept in shell vials (95 mm height, 30 mm diameter) with a standard cornmeal food mix (cornmeal, agar, corn syrup, yeast, tegosept, propionic acid, and phosphoric acid).

### Mating Scheme

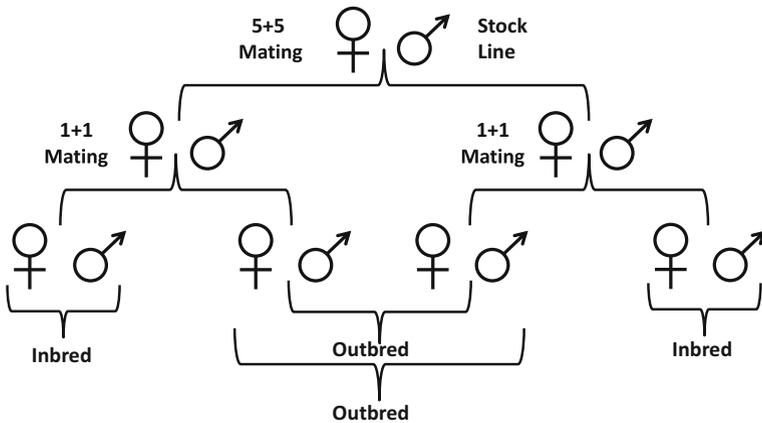
From each of the stock lines, 5+5 matings (where 5 males and 5 females were put in a vial and allowed to mate at will) were performed in order to procure a large number of virgin flies. Randomly chosen virgins from these 5+5 matings

were used in 1+1 matings (where 1 male and 1 female are put into a vial and allowed to mate) in two parallel sublimes. Virgins collected from these 1+1 matings were then used to create inbred and outbred individuals within each subline, (see Fig. 1). The male and female individuals paired for the mate choice assay (see below) were from opposing sublimes and were therefore not directly related.

### Imaging and Identification of Male Flies

In order to be able to identify which males were chosen by the female, the wings of the males were imaged using a system very similar to the WINGMACHINE system (Houle et al. 2003). Briefly, the wings of males were held between a glass slide and a slide cover by a vacuum and a photograph was taken of the wing under a microscope and captured on a computer. The computer overlaid a spline onto the image in order to calculate the distances between intersections of the veins in the wings. This data has been used successfully to quantitatively describe the size and shape of *Drosophila* wings (Carter et al. 2009a, b; Carter and Houle 2011; Pelabon et al. 2010).

Imaging was performed before and after the mate choice assays occurred. Measurements from the second set of images were compared to the first set to identify the male chosen. Due to extreme conservation in wing shape, overall size (centroid size based on the 8 distal landmarks in the wing) was primarily used to identify the flies used. In cases in which an unambiguous identification could not be made, that mating was omitted from the analysis.



**Fig. 1** Mating Scheme used to generate relatively inbred and outbred individuals. Two sublimes were used to generate the flies used in this experiment; the mating scheme used in each is shown. Matings of five males and five females from a large base population were used to generate virgins for use in the second mating step. Single male and female matings were performed to produce virgins for the third mating step. In the third mating step inbred individuals come from mating siblings while relatively outbred individuals come from mating unrelated virgins. The figure depicts the generation of inbred and outbred individuals within a *single subline*; the mate choice experiments paired individuals from *opposite sublimes* to remove any effect of relatedness instead of homozygosity. This mating scheme was used for each of the *three lines* studied: Irene, Jon, and Rose

## Mate Choice Experiment

The mate choice device consisted of a standard vial with a plastic divider down the center separating the vial into two regions. Two males (one inbred and one outbred) were put on one side of the divider and one female (either inbred or outbred) from the other subline was put on the other side. No female was presented with a directly related male during the mate choice experiment. They were then given a 24 h recovery period from the CO<sub>2</sub> anesthetization. After this recovery period, the divider was removed, allowing the female and males to associate, and the flies were watched to determine when a mate was chosen (when copulation began). At this point, the flies were anesthetized and the males were separated and stored in individual vials labeled as single or mated. The time taken to choose the male was also recorded. Males were reimaged within 5 days for identification.

In order to control for the possibility that female choice may be influenced by male size, males from each of the treatments were sorted by size in opposite directions (i.e., males were arranged from largest to smallest in one treatment and then paired against males arranged from smallest to largest in the other) so that approximately half of the vials contained inbred males that were larger than the outbred males and the other half had larger outbred males.

## Statistics

For each of the six trials, two-tailed binomial tests were performed to determine significance of the frequencies with which inbred and outbred males were selected. Differences in preference are presented using the odds ratio, OR. Within each of the three lines the Chi-squared test, G test and Fisher's exact test were used to test for differences in frequencies of choices between the two female treatments and a Mantel-Haenszel test was performed on the combined data set. Individual heteroscedastic student's *t* tests and the General Linear Model (GLM) module in Minitab 16 were used to analyze the natural log transformed values of the times to mate to test for differences in the time taken for choices to occur in the trials and to identify significant factors.

## Results

All six combinations of line and female treatments showed a trend toward preference for outbred males (Table 1, Fig. 2) although only the outbred Jon female treatment showed a significant preference individually ( $p=0.0093$ , two-tailed binomial test). The binomial probability that all six lines would show the same pattern under a null hypothesis of no overall preference would be  $p=0.031$  ( $p=0.016$  if we assume an a priori expectation of preference for outbred males) and a data set made from combining all six sets of trials is highly significant ( $p=0.0027$ , two-tailed binomial test) so we therefore consider the overall observed pattern of preferences for outbred males over inbred ones to be significant.

Within each line the preference appeared more pronounced in outbred females than in inbred ones (odds ratio, OR=1.21, 1.46, 1.04 for the Irene, Jon and Rose lines

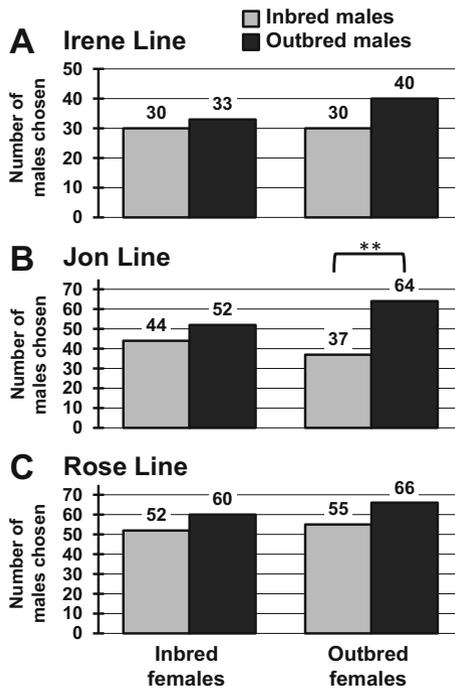
**Table 1** Results of mate choice experiment. For each of the two female treatments and the combined data set for each of the three lines the number of choices of each type made by the females is shown. For the choices of inbred versus outbred males, in all six treatments females chose outbred males more than inbred males. For the choices of larger versus smaller males, in three of the six treatments females chose larger males more than smaller males

Line	Female Treatment	# Inbr. vs Outbr. Males	# Smaller vs Larger Males
Irene	Inbred	30 vs 33	32 vs 31
	Oubred	30 vs 40	27 vs 43 <sup>a</sup>
	Combined	60 vs 73	59 vs 74
Jon	Inbred	44 vs 52	54 vs 42
	Oubred	37 vs 64 <sup>b</sup>	47 vs 54
	Combined	81 vs 116 <sup>a</sup>	101 vs 96
Rose	Inbred	52 vs 60	61 vs 51
	Oubred	55 vs 66	60 vs 61
	Combined	107 vs 126	121 vs 112

Significant differences revealed by a binomial test of these values are indicated with:

<sup>a</sup> when  $p < 0.05$  and

<sup>b</sup> when  $p < 0.01$



**Fig. 2** Female Mate Choice preferences. The number of times inbred and outbred males were chosen by females is shown organized by the inbred or outbred status of the female within each of the lines. **a** Irene line, **b** Jon Line, **c** Rose line

respectively), but these differences were not significant individually using either  $X^2$ , G test or Fisher's exact test methods ( $p > 0.05$  for all tests) and a Mantel-Haenszel test incorporating the three sets as strata indicated likewise ( $X^2_2 = 1.097$ ,  $p = 0.295$ ).

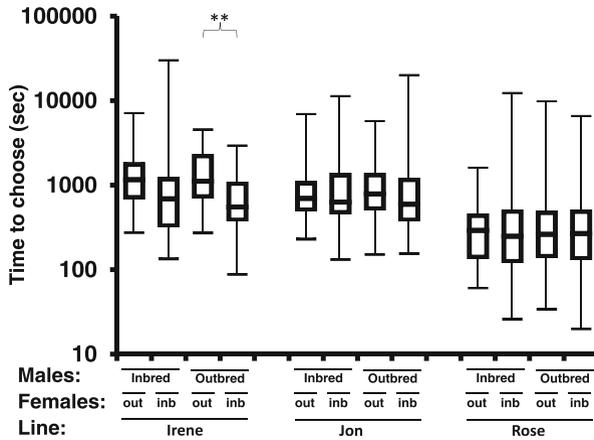
There was a non-significant trend in the sizes of the inbred and outbred males used in the mate choice experiment within each line (unpaired heteroscedastic t tests:  $t_{333} = 0.785$ ,  $p = 0.433$ ;  $t_{490} = 0.881$ ,  $p = 0.379$ ;  $t_{697} = 1.507$ ,  $p = 0.133$  for the Irene, Jon and Rose lines respectively), but in all three lines the mean inbred male size was less than the mean outbred male size which is consistent with slight inbreeding depression.

When testing the choices for larger vs. smaller male in all six treatments there was no overall trend in preference for larger or smaller males; three of the six combinations showed a non-significant preference in each direction (Table 1), with only the outbred Irene female treatment showing a significant preference individually ( $p = 0.036$ , two-tailed binomial test). Within each line the preference appeared more pronounced in outbred females than in inbred ones (OR = 1.64, 1.48, 1.22 for the Irene, Jon and Rose lines respectively), but these differences were not significant individually using either  $X^2$  G test or Fisher's exact test methods ( $p > 0.05$  for all tests), however a Mantel-Haenszel test incorporating the three sets as strata indicated support for a stronger size preference in the outbred females than the inbred ones ( $X^2_2 = 9.260$ ,  $p = 0.0023$ ).

The distribution of the times taken by females to make their choices was highly positively skewed with most choices occurring within 10–15 min while a small number of females took up to several hours (Fig. 3). We therefore analyzed the natural log transformed values of the time measurements. A general linear model (GLM) analysis indicated significant line ( $F_{2,492} = 79.98$ ,  $p < 0.001$ ) and female inbreeding status ( $F_{1,492} = 4.66$ ,  $p = 0.031$ ) factors, but male status and all pairwise interactions of factors were non-significant (male status  $F_{1,492} = 0.00$ ,  $p = 0.964$ ; line X female status  $F_{2,492} = 2.90$ ,  $p = 0.056$ ; line X male status  $F_{2,492} = 0.17$ ,  $p = 0.842$ ; female status X male status  $F_{1,492} = 0.03$ ,  $p = 0.854$ ). When considering the six female treatments individually, there was a weak overall trend in the transformed mean of the times taken by females to choose with five of the six female treatments showing choices of inbred males to occur more quickly, but these differences were not significant individually or overall (unpaired heteroscedastic t tests:  $p > 0.05$  for all tests). When considering the six male treatments individually there was a weak overall trend in the mean of the times taken to choose with five of the six treatment combinations showing faster choices by inbred females with these differences only significant in one line (inbred females choose more quickly than outbred females in the outbred male Irene treatment, heteroscedastic student's *t* test;  $t = 2.97$ ,  $df = 55.6$ ,  $p = 0.0022$ ;  $p > 0.05$  for all other male treatments), but the GLM analysis indicated that this overall pattern was significant.

## Discussion

In this experiment, more outbred males were chosen than inbred ones in all six treatments which is a significant pattern and this difference was also significant within one of the treatments (Table 1, Fig. 2). This pattern is consistent with results reported elsewhere showing preference for more heterozygous males (e.g., Ilmonen et al. 2009; Bolund et al. 2010; Zajitschek and Brooks 2010; Pölkki et al. 2012). Our results also suggest that outbred females may have a



**Fig. 3** Time to mate choice. The number of seconds taken by females to choose males is shown for the four combinations of treatments within each line. None of the individual combinations of treatments within each male or female treatment was significantly different from the others except the time taken to choose outbred males by inbred females was significantly less than the time taken by outbred females in the Irene line ( $p=0.0022$ ; two-tailed student’s heteroscedastic  $t$  test of Natural Log transformed values). A general linear model (GLM) analysis indicated significant line ( $p<0.001$ ) and female inbreeding status ( $p=0.031$ ) factors, but male status and all pairwise interactions of factors were non-significant ( $p>0.05$ )

more pronounced mating preference for outbred males than inbred females although these results were not significant. This observed pattern is in agreement with the stronger preference for heterozygous males exhibited by heterozygous females in blue tits (*Cyanistes caeruleus*) (García-Navas et al. 2009), but contrary to the study by Griggio et al. (2011) that indicated a lack of preference for heterozygosity by heterozygous house sparrow females (*Passer domesticus*). Outbred females showed a non-significant preference for larger males while inbred females exhibited an opposite non-significant pattern and the difference in these preferences was significant.

Consideration of the mean time taken to choose indicated that the inbred females made their choices more quickly than the outbred females, which is often taken to be indicative of stronger preferences (e.g., Dukas 2005; Taylor et al. 2007). This pattern was seen in 5 of 6 male treatments, with a significant difference in the time to choose seen within one treatment (outbred males in the Irene line), and the overall analysis indicated significant effects for line and female inbreeding status while male status and all pairwise interactions were non-significant. Our data therefore indicates that the less selective inbred females made their choices more quickly than the more selective outbred females did which is interesting as this is opposite to the pattern usually assumed (e.g., Dukas 2005; Taylor et al. 2007).

We consider the totality of our results to provide evidence that the females preferred outbred males and show non-significant trends suggesting that this preference may be more pronounced in outbred females. There was no overall pattern of preference for larger size, but outbred females did appear to show a stronger preference for larger size than inbred females when all data was combined. Differences in the preferences of inbred and outbred females may arise for several reasons and we consider three

possible causes of the trend consistent with differences in mate choice behaviors we observed below.

First, inbred females, likely to have a lower fitness as indicated by the trend toward smaller size seen in the inbred treatment males, may have a more difficult time distinguishing inbred males from outbred males due to a poorer ability to assess males rather than preference per se. In a study of *Drosophila melanogaster*, inbred flies were less responsive to training to avoid certain odors than outbred ones and this effect may have arisen from reduced olfactory ability (Nepoux et al. 2010). If females that are less perceptive make faster decisions the trend in the time taken for inbred and outbred females to choose is consistent with this hypothesis. On the other hand, if females that are less able to distinguish quality take longer periods of time to assess their potential mate in order to get more information, we would expect inbred females to take significantly longer to choose than outbred females. The result that outbred females take longer to choose is not consistent with this second mechanism.

Second, a number of factors appear to influence female choice (Jennions and Petrie 1997), including self-referential behaviors. Outbred females may recognize their own higher fitness (or higher value as a mate) and be more meticulous in their choice of mate, as seems to be the case for blue tits (*Cyanistes caeruleus*) (García-Navas et al. 2009). Conversely, inbred females may recognize their own lower fitness (lower value as a mate) and be less meticulous. In zebra finches (*Taeniopygia guttata*), lower choosiness was seen in inbred females (Bolund et al. 2010) and females manipulated to have reduced condition (Burley and Foster 2006). One way females may have recognized their own relative fitness is by observing the reactions of males towards them (Bolund et al. 2010; Tien et al. 2011). The non-significant trends toward stronger preferences seen in the outbred females relative to the inbred ones and the increased times taken to make these decisions are consistent with this hypothesis.

Third, a possible cause for the inbred females showing less of a preference to avoid homozygous mates is that some of the flies, due to the slight inbreeding, became homozygous for advantageous alleles that gave an extra advantage when they were together. Breaking up these homozygous alleles would decrease the fitness of the individual in which they were broken up. In a perennial herb (*Anchusa crispera*), inbred lines did better when they continued to inbreed; they showed outbreeding depression more quickly than inbreeding depression (Quilichini et al. 2001). A comparison of a number of bumble bee (*Bombus terrestris*) colonies with varying degrees of inbreeding indicated a mix of outbreeding and inbreeding depression with indications that in at least some cases inbreeding was advantageous (Gerloff and Schmid-hempel 2005). Perhaps in some cases the inbred female flies were choosing inbred flies whose homozygous alleles were similar to their own, thereby preventing the breakup of those alleles in their progeny.

In contrast to our results, many recent studies have rejected the idea that increased heterozygosity leads to female mate choice preference. One study in schistosomes (*Schistosoma mansoni*) found that genetic dissimilarity positively influenced divorce rate, but male heterozygosity did not (Beltran et al. 2008). A study on flour beetles (*Tribolium castaneum*) found no evidence that females chose mates based on heterozygosity (Pai and Yan 2002). A study in white-rumped swallows (*Tachycineta leucorrhoa*) found that nests with extra-pair young had a higher overall survival rate than nests with only within-pair young; this was unexpected because the nest mate was

less genetically similar to the female than her extra-pair partner and highly heterozygous fledglings had a lower probability of survival (Ferretti et al. 2011). Potentially contributing to these mixed results is the observation that many studies that work on heterozygosity and mate choice that use molecular methods to assess overall heterozygosity do not use a large enough number of loci, which may not confer enough power to see the effects of heterozygosity (Smith et al. 2005). Additionally, the presence of statistical biases in many uses of observed heterozygosity values in studies of mate choice behavior may be prevalent because of the use of the same loci for heterozygosity estimation and paternity assignment (Wetzel and Westneat 2009). The design of our experiment avoids these potential biases.

Although there have been many studies on the effects of inbreeding on mate choice; in many of these, especially in *Drosophila*, the subject for study is extremely inbred or artificially inbred. For example, a study on *Drosophila melanogaster* showed that males who were completely homozygous at chromosome 2 (through the use of balancer chromosomes) had reduced ability to court females, and to sire offspring than heterozygous males (Miller et al. 1993), but this extreme treatment may not accurately reflect natural circumstances. In nature, organisms are likely to be only slightly inbred rather than completely homozygous for entire chromosomes. In our study, the relatively inbred flies were only inbred for one generation, a scenario much more likely to occur in nature. Although this relatively mild treatment made any mate choice preferences more difficult to detect than with more extreme treatments, an overall preference for males with higher levels of heterozygosity was still detected under these conservative conditions. Our data suggests that in natural circumstances, flies will have a preference towards avoiding inbred partners independent of a general inbreeding avoidance based on avoiding close relatives.

This study suggests that females of *Drosophila melanogaster* and *Drosophila simulans* have a preference for heterozygosity in males and that outbred females may exhibit stronger mate choice preferences than relatively inbred ones. In addition to the interest inherent in elucidating a component of mate choice behavior in natural populations, these results may also be of use in understanding factors that influence the success of captive breeding programs. Our results suggest that attention be paid not just to the relatedness of individuals in such programs, but also to the degree of heterozygosity of each individual as this may influence their expected willingness to mate or be chosen as mates. The exact mechanism by which these differences in preference is made possible (e.g., behavioral or hormonal differences in the males) is unknown and further research is required to determine the mechanisms used for inbreeding or outbreeding avoidance and determine the overall frequency with which these occur. We note that while there are a number of studies on the effects of inbreeding, there are comparatively few on outbreeding and degree of relatedness. Until the effects of both inbreeding and outbreeding on mate choice are understood better, understanding the overall degree to which each influences mate choice will remain unresolved.

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## References

- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Aparicio JM, Cordero PJ, Veiga JP (2001) A test of the hypothesis of mate choice based on heterozygosity in the spotless starling. *Anim Behav* 62:1001–1006
- Beltran S, Cézilly F, Boissier J (2008) Genetic dissimilarity between mates, but not male heterozygosity, influences divorce in schistosomes. *PLoS ONE* 3:e3328
- Bolund E, Martin K, Kempnaers B, Forstmeier W (2010) Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Anim Behav* 79:947–955
- Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G (2006) Complex Mhc-based mate choice in a wild passerine. *Proc R Soc B* 273:1111–1116
- Brown JL (1996) A theory of mate choice based on heterozygosity. *Behav Ecol* 8:60–65
- Burley NT, Foster VS (2006) Variation in female choice of mates: condition influences selectivity. *Anim Behav* 72:713–719
- Carter AJR, Houle D (2011) Artificial selection reveals heritable variation for developmental instability. *Evolution* 65:3558–3564
- Carter AJR, Osborne E, Houle D (2009a) Heritability of directional asymmetry in *Drosophila melanogaster*. *Int J Evol Biol* 2009:759159
- Carter AJR, Weier TM, Houle D (2009b) The effect of inbreeding on fluctuating asymmetry of wing veins in two laboratory strains of *Drosophila melanogaster*. *Heredity* 102:563–572
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst* 18:237–268
- Darwin CR (1871) The descent of man and selection in relation to sex. John Murray, London
- Darwin CR (1876) The effects of cross and self fertilization in the vegetable kingdom. John Murray, London
- De Luca PA, Cocroft RB (2008) The effects of age and relatedness on mating patterns in thornbug treehoppers: inbreeding avoidance or inbreeding tolerance? *Behav Ecol Sociobiol* 62:1869–1875
- Dukas R (2005) Learning affects mate choice in female fruit flies. *Behav Ecol* 16(4):800–804
- Ferretti V, Massoni V, Bulit F, Winkler DW, Lovette IJ (2011) Heterozygosity and fitness benefits of extrapair mate choice in white-rumped swallows. *Behav Ecol* 6:1178–1186
- Fox CW, Xu J, Wallin WG, Curtis CL (2012) Male inbreeding status affects female fitness in a seed-feeding beetle. *J Evol Biol* 25:29–37
- Frommen JG, Bakker TCM (2006) Inbreeding avoidance through non-random mating in sticklebacks. *Biol Lett* 2:232–235
- García-Navas V, Ortego J, Sanz JJ (2009) Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. *Proc R Soc B* 276:2931–2940
- Gerloff CU, Schmid-hempel P (2005) Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera: Apidae). *Oikos* 1:67–80
- Griggio M, Biard C, Penn DJ, Hoi H (2011) Female house sparrows “count on” male genes: experimental evidence for MHC-dependent mate preference in birds. *BMC Evol Biol* 11:44
- Guevara-Fieore P, Rosenqvist G, Watt PJ (2010) Inbreeding level does not induce female discrimination between sibs and unrelated males in guppies. *Behav Ecol Sociobiol* 64:1601–1607
- Hoffman JI, Forcada J, Trathan PN, Amos W (2007) Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* 445:912–914
- Houle D, Mezey J, Galpern P, Carter AJR (2003) Automated measurement of *Drosophila* wings. *BMC Evol Biol* 3:25
- Ilmonen P, Stundner G, Thoss M, Penn DJ (2009) Females prefer the scent of outbred males: good-genes-as-heterozygosity? *BMC Evol Biol* 9:104
- Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72:283–327
- Kempnaers B (2007) Mate choice and genetic quality: a review of the heterozygosity theory. *Adv Study Behav* 37:189–278
- Kokko H, Ots I (2006) When not to avoid inbreeding. *Evolution* 60:467–475
- Laloi D, Eizaguirre C, Fédérici P, Massot M (2011) Female choice for heterozygous mates changes along successive mating in a lizard. *Behav Process* 88:149–154
- Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland
- Marques I, Feliner GN, Martins-Loução MA, Aguilar JF (2011) Fitness in *Narcissus* hybrids: low fertility is overcome by early hybrid vigour, absence of exogenous selection and high bulb propagation. *J Ecol* 99: 1508–1519

- Masters KL, Masters AR, Forsyth A (1994) Female-biased sex ratios in the neotropical treehopper *Umbonia ataliba* (Homoptera: Membracidae). *Ethology* 96:353–366
- Mazzi D, Künzler R, Largiadèr CR, Bakker TCM (2004) Inbreeding affects female preference for symmetry in computer-animated sticklebacks. *Behav Genet* 34:417–424
- Miller RS, Glasner J, Hedrick RW (1993) Inbreeding depression and male-mating behavior in *Drosophila melanogaster*. *Genetica* 88:29–36
- Mueller LD, Joshi A, Santos M, Rose M (2013) Effective population size and evolutionary dynamics in outbred laboratory populations of *Drosophila*. *J Genet* 92(3):349–361
- Nepoux V, Haag CR, Kawecki TJ (2010) Effects of inbreeding on aversive learning in *Drosophila*. *J Evol Biol* 23:2333–2345
- Pai A, Yan G (2002) Female mate choice in relation to heterozygosity in *Tribolium castaneum*. *J Evol Biol* 15: 1076–1082
- Palmer CA, Edmands S (2000) Mate choice in the face of both inbreeding and outbreeding depression in the intertidal copepod *Tigriopus californicus*. *Mar Biol* 136:693–698
- Pelabon C, Hansen TF, Carter AJR, Houle D (2010) Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection. *Evolution* 64(7):1912–1925
- Pölkki M, Krams I, Kangassalo K, Rantala MJ (2012) Inbreeding affects sexual signaling in males but not females of *Tenebrio molitor*. *Biol Lett* 8:423–425
- Quilichini A, Debussche M, Thompson JD (2001) Evidence for local outbreeding depression in the Mediterranean island endemic *Anchusa crisper Viv.* (Boraginaceae). *Heredity* 87:190–197
- Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* 414:300–302
- Ryder TB, Tori WP, Blake JG, Loiselle BA, Parker PG (2009) Mate choice for genetic quality: a test of the heterozygosity and compatibility hypotheses in a lek-breeding bird. *Behav Ecol* 21:203–210
- Seko T, Miyatake T, Miura K (2012) Assessment of hybrid vigor between flightless lines to restore survival and reproductive characteristics in the ladybird beetle *Harmonia axyridis*. *BioControl* 57:85–93
- Shahid MQ, Hai-Ming X, Shun-Quan L, Zhi-Xiong C, Naeem M, Ya-Juan L, Xiang-Dong L (2012) Genetic analysis and hybrid vigor study of grain yield and other quantitative traits in autotetraploid rice. *Pak J Bot* 44(1):237–246
- Sherman CDH, Wapstra E, Uller T, Olsson M (2008) Males with high genetic similarity to females sire more offspring in sperm competition in Peron's tree frog *Litoria peronii*. *Proc R Soc B* 275:971–978
- Smith SB, Webster MS, Holmes RT (2005) The heterozygosity theory of extra-pair mate choice in birds: a test and a cautionary note. *J Avian Biol* 36:146–154
- Suvanto L, Liimatainen JO, Tregenza T, Hoikkala A (2000) Courtship signals and mate choice of the flies of inbred *Drosophila montana* strains. *J Evol Biol* 13:583–592
- Tan CKW, Løvlie H, Pizzari T, Wigby S (2012) No evidence for precopulatory inbreeding avoidance in *Drosophila melanogaster*. *Anim Behav* 83:1433–1441
- Taylor ML, Wedell N, Hosken DJ (2007) The heritability of attractiveness. *Curr Biol* 17(22):R959–R960
- Thünken T, Baldauf SA, Kullmann H, Schuld J, Hesse S, Bakker TCM (2011) Size-related inbreeding preference and competitiveness in male *Pelvicachromis taeniatus* (Cichlidae). *Behav Ecol* 22:358–362
- Tien NSH, Massourakis G, Sabelis MW, Egas M (2011) Mate choice promotes inbreeding avoidance in the two-spotted spider mite. *Exp Appl Acarol* 54:119–124
- Välimäki P, Kivelä SM, Mäenpää MI (2011) Mating with a kin decreases female remating interval: a possible example of inbreeding avoidance. *Behav Ecol Sociobiol* 65:2037–2047
- Viken A, Fleming I, Rosenqvist G (2006) Premating avoidance of inbreeding absent in female guppies (*Poecilia reticulata*). *Ethology* 112:716–723
- Vorsino AE, Wiczorek AM, Wright MG, Messing RH (2012) An analysis of heterosis and outbreeding depression among lab-reared populations of the parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae); potential implications for augmentative release. *Biol Control* 61:26–31
- Wetzel D, Westneat D (2009) Heterozygosity and extra-pair paternity: biased test results from the use of shared markers. *Mol Ecol* 18:2010–2021
- Zajitschek SRK, Brooks RC (2010) Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata*. *Behav Ecol* 4:884–891