

ASSOCIATION BETWEEN SEX RATIO DISTORTION AND SEXUALLY ANTAGONISTIC FITNESS CONSEQUENCES OF FEMALE CHOICE

Tim Connallon^{1,2} and Erin Jakubowski¹

¹Department of Ecology & Evolutionary Biology and Museum of Zoology, University of Michigan, Ann Arbor 48109

²E-mail: tconnal@umich.edu

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Genetic variation can be beneficial to one sex yet harmful when expressed in the other—a condition referred to as sexual antagonism. Because X chromosomes are transmitted from fathers to daughters, and sexually antagonistic fitness variation is predicted to often be X-linked, mates of relatively low-fitness males might produce high-fitness daughters whereas mates of high-fitness males produce low-fitness daughters. Such fitness consequences have been predicted to influence the evolution of female mating biases and the offspring sex ratio. Females might evolve to prefer mates that provide good genes for daughters or might adjust offspring sex ratios in favor of the sex with the highest relative fitness. We test these possibilities in a laboratory-adapted population of *Drosophila melanogaster*, and find that females preferentially mate with males carrying genes that are deleterious for daughters. Preferred males produce equal numbers of sons and daughters, whereas unpreferred males produce female-biased sex ratios. As a consequence, mean offspring fitness of unpreferred males is higher than offspring fitness of preferred males. This observation has several interesting implications for sexual selection and the maintenance of population genetic variation for fitness.

KEY WORDS: Cryptic female choice, good genes, intralocus sexual conflict, X chromosome.

The presence of sexually antagonistic variation—where alleles increasing male fitness are deleterious when expressed in female genomes—poses a dilemma for the evolution of female choice (Chippindale et al. 2001). Females face a trade-off of producing relatively fit sons and unfit daughters, or relatively fit daughters and unfit sons. If sexually antagonistic variation is sufficiently abundant and X-linked, as suggested by theory and data (Rice 1984; Gibson et al. 2002; Pischedda and Chippindale 2006), there will be no benefit of mating with relatively fit males, but there can be a cost associated with the production of low-fitness daughters.

Two possible evolutionary responses are predicted when sexually antagonistic fitness variation predominates, and has a strong X-linked component. Albert and Otto (2005) argued that female mating biases will evolve to favor males providing good genes to daughters. Female choice might therefore reverse the direction of selection acting on males and resolve the sexual antagonism. Calsbeek and Sinervo (2004) proposed an alternative response, that

females should modify the sex ratio of their offspring to minimize indirect costs of mating with relatively high-fitness males.

The fruit fly *Drosophila melanogaster* is a suitable species for testing these hypotheses. Laboratory-adapted populations of flies, where the environmental context of adaptation is known, are amenable to the measurement of traits closely associated with fitness. Previous studies show that sexually antagonistic genetic variation influences adult fitness variation (Chippindale et al. 2001; Long and Rice 2007), and much of this variation appears to be X-linked (Gibson et al. 2002; Pischedda and Chippindale 2006). Furthermore, females can adjust the sex ratio of their offspring (Mange 1970; Long and Pischedda 2005; Fuller and Mousseau 2007), suggesting that adaptive sex ratio adjustment with respect to sexually antagonistic variation is at least possible.

We conducted experiments using a laboratory-adapted population of fruit flies to address two questions: (1) Do female mating

biases lead to sexually antagonistic fitness consequences for offspring? (2) Is the offspring sex ratio skewed in favor of the sex with the highest relative fitness?

Methods

DROSOPHILA STOCKS

Female choice and adult fitness components were estimated using the IV population, a laboratory-adapted population of *D. melanogaster* that is described in Houle and Rowe (2003). The IV population and the competitor population, IVe—a laboratory-adapted population that is homozygous for the *ebony* mutation—were kindly provided by David Houle.

MATE CHOICE TRIALS

Female mating biases were ascertained by two approaches. A series of tournament-style mating trials were used to identify males differing in mating success (Fig. S1). Trials used two- and three-day-old virgin males and females, sampled from the IV population. Each trial was conducted with a pair of males and a single virgin female. The first male to successfully mate was designated as the winner. Winners or losers from each round of trials were arbitrarily paired with each other and retested. Males that lost or won three successive trials were used for sex ratio and offspring fitness assays.

Male–male competition, or intrasexual selection, might influence the outcome of the tournament-style assays, and could potentially override female mating biases. To test whether females preferentially mate with males that perform well in the tournament setting, we conducted a series of female mating latency experiments. A single male and a virgin female were placed in a vial and observed until copulation occurred. The relative success of each male was estimated by the time required for him to mate. Successful males from the tournament trials were able to achieve copulation more quickly than unsuccessful males (Fig. S2), indicating that the tournament trials capture information about female mating biases and are not driven by male–male competition. Females bias matings toward relatively successful (3 wins, 0 losses) and away from relatively unsuccessful (3 losses, 0 wins) males. Such males are hereafter referred to as “preferred” and “unpreferred,” respectively.

OFFSPRING SEX RATIO AND ADULT FITNESS COMPONENTS

During the day after mating success trials were concluded, preferred and unpreferred males were assigned at random to virgin females from the IV population. Previous research using *D. melanogaster* indicates that male fertility quickly recovers during a lag period of this duration (Markow et al. 1978), and thus,

seminal fluid limitation should not adversely affect the mates of preferred males. Mates of experimental males were then permitted to lay eggs in vials for 12 h. From these eggs, sets of 40 to 50 eggs were transferred to 8-ounce bottles, each containing standard cornmeal medium, 20 adult *ebony* females, and 20 *ebony* males. This is a typical adult density for IV and IVe flies, creating a typical larval environment for this laboratory-adapted population. The relatively low density of introduced eggs per bottle also minimizes interactions between the experimental offspring.

Offspring sex ratio was examined in three independent experimental trials. The first two trials each followed offspring of 40 preferred and 40 unpreferred males. The third trial followed offspring of 70 preferred and 70 unpreferred males. Offspring from the third trial were used for fitness assays. Adult offspring were collected on the 14th day, consistent with the evolutionary history of the population, which has been continuously reared on a 14-day generation cycle since 1975, representing over 800 generations of adaptation to the laboratory environment (Houle and Rowe 2003).

Sex-specific selection in *D. melanogaster* may influence the evolution of juvenile growth traits (Prasad et al. 2007). Juvenile growth differences can therefore potentially underlie sexually antagonistic fitness effects that manifest at the adult stage. However, juvenile sex-specific selection does not appear to give rise to sexually antagonistic viability selection. Indeed, there is a strong, positive intersexual genetic correlation for *Drosophila* juvenile viability (e.g., Chippindale et al. 2001). Because our major concern here is with sexual antagonism, the results focus on adult fitness-related traits—female fecundity and male mating success—that are potentially influenced by sexually antagonistic variation. However, the overall conclusions do not rely upon an emphasis on adult-stage fitness. Estimates of egg-to-adult viability for preferred and unpreferred males reveal no mortality differences between treatments (unpreferred offspring: $n = 1248$ eggs, 70.8% survival; preferred offspring: $n = 1082$ eggs, 69.8% survival; two-tailed Fisher’s exact test $P = 0.82$).

Adult-stage female fitness was estimated by the number of eggs produced on the 14th day of the life cycle. Female offspring were placed in pairs, along with two randomly assigned *ebony* males, into vials containing standard cornmeal medium and were permitted to lay eggs for 24 h. Houle and Rowe (2003) previously showed that this is the critical time period during which egg laying contributes to adult female fitness.

Male fitness was estimated by mating success experiments, conducted during the 14th day of the life cycle. Male offspring were individually transferred to mate competition vials, each containing a randomly selected (and unrelated) male and female. These females and competitor males were each heterozygous for an *ebony* allele, and expressed the wild-type pigmentation pattern. Each competition vial was observed until the female mated with

one of the males. The female was then isolated and permitted to lay eggs in a fresh vial. Paternity was assigned 14 days later by the presence or absence of *ebony*-expressing offspring. This measure of male fitness eliminates postcopulatory sexual selection, but is not expected to bias the results because precopulatory mating success is positively correlated with sperm competition success (Bangham et al. 2002; Hosken et al. 2008; although the two traits have different genetic bases: Zhang et al. 2008). The measure also assumes that mating success on day 14 is correlated with overall male success, which will be a function of success on or before day 14. This assumption could potentially be violated if there is an extreme reversal in relative male mating success during the span of a couple of days, but there is no a priori reason to expect such an extreme reversal, nor is there any such precedent in *D. melanogaster*.

Chi-square tests were used to detect sex ratio deviations from unity (1:1). Fisher's exact tests were used to examine whether preferred and unpreferred fathers had sons with different degrees of mating success. Two-tailed *t*-tests were used to test whether egg production rates differed between daughters of preferred and unpreferred fathers.

Results and Discussion

OFFSPRING FITNESS ESTIMATES

Preferred fathers had sons with slightly higher mating success, although the difference was small and not statistically significant (percent paternity unpreferred = 0.627, $n = 193$; percent paternity preferred = 0.647, $n = 207$; Fisher's exact test $P = 0.679$). Daughters of preferred males had decreased fecundity compared to the daughters of unpreferred males (Fig. 1; unpreferred daughters produced a mean of 70.20 eggs per vial, $n = 203$ vials; preferred daughters produced 56.85 eggs per vial, $n = 164$ vials; two-tailed *t*-test $P = 0.0000004$). The estimated fitness gain of 3% to sons is substantially lower than the estimated fitness drop of 19% to daughters of preferred males.

A strong, negative fitness correlation between fathers and daughters, coupled with marginal father-son fitness heritability cannot be explained by autosome-linked sexually antagonistic variation, which predicts that costs and benefits will be symmetrical between sons and daughters (Kidwell et al. 1977). It is also possible that females differentially provision eggs fertilized by unpreferred males. However, a "paternal effect" such as this should reduce the fitness of both sons and daughters—this prediction is difficult to reconcile with the data. The offspring fitness pattern is consistent with prior theory and data suggesting that adult fitness traits are strongly influenced by X-linked sexually antagonistic variation (Rice 1984; Gibson et al. 2002; Pischedda and Chippindale 2006; Oneal et al. 2007).

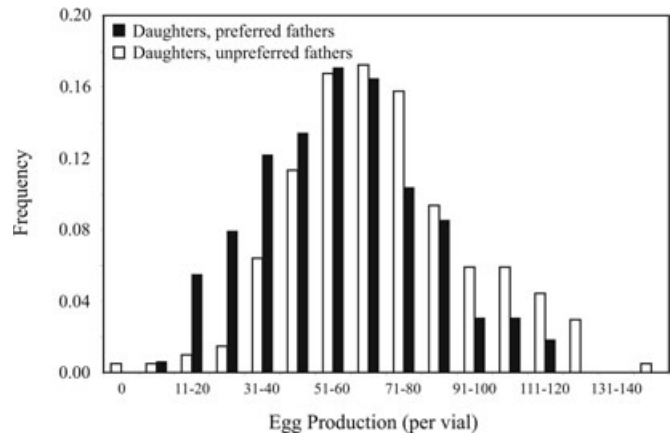


Figure 1. Paternal mating success influences daughter fecundity. The egg production distribution is based on egg counts per vial, each containing two experimental females and two randomly selected males (see Methods for details).

OFFSPRING SEX RATIO

Offspring sex ratios differed between experimental treatments, with preferred males producing sons and daughters at equal ratios, and unpreferred males producing daughter-biased sex ratios (Table 1). The single exception in trial 2 (equal sex ratio for preferred and unpreferred fathers) can be attributable to its markedly reduced sample size relative to sampling in trials 1 and 3. Indeed, the overall sex ratio reduction in unpreferred male broods is relatively strong and highly significant (male:female ratio = 0.874; $P = 0.0056$).

There are three possible mechanistic explanations for the sex ratio biases observed here. The sex ratio might be equal among fertilized eggs, but viability selection might differentially remove males and females from the adult population. This mechanism would require that son and daughter viability be decoupled for

Table 1. Male mating success and offspring sex ratios.

	Males	Females	Sex ratio	P^1
Trial 1				
Unpreferred	296	366	0.809	0.0065
Preferred	255	260	0.981	0.83
Trial 2				
Unpreferred	78	72	1.08	0.62
Preferred	87	86	1.01	0.94
Trial 3				
Unpreferred	417	467	0.893	0.093
Preferred	377	378	0.997	0.97
All trials				
Unpreferred	791	905	0.874	0.0056
Preferred	719	724	0.993	0.90

¹Significance based on chi-squared tests with one degree of freedom. Statistically significant values are in bold.

unpreferred but not for preferred males. This scenario appears unlikely in *D. melanogaster*, where juvenile viability is strongly positively correlated between the sexes (Chippindale et al. 2001).

Females might adjust progeny sex ratios in response to their mate. This is a possibility in *D. melanogaster*, where females have been shown to adjust offspring sex ratio in a mating context-dependent manner (Mange 1970; Long and Pischedda 2005; Fuller and Mousseau 2007). When variation is sexually antagonistic, this hypothesis predicts that females mated to preferred males will produce male-biased offspring sex ratios whereas unpreferred males will sire female-biased broods. Only part of this pattern is supported, as preferred male broods have a sex ratio near unity. Nevertheless, the direction of skew in offspring of unpreferred males is adaptive because it is biased toward the sex with highest relative fitness (daughters).

Males with low mating success might have sex ratio distorting X chromosomes, which are common in *Drosophila* populations (Jaenike 2001). Associations between mating success and male meiotic drive have been reported in studies using mice and stalk-eyed flies (Lenington 1991; Wilkinson et al. 1998), although it is not known whether “driving” X chromosomes are associated with sexually antagonistic variation in these species. Such linkage disequilibrium might be expected. Males carrying a driving X with female-beneficial alleles will have higher-quality offspring than males carrying driving, female-detrimental X chromosomes. The effect could promote the development of linkage disequilibrium between sex ratio and sexually antagonistic alleles. This is an area of population genetics theory that is relatively unexplored mathematically (Rice 1986; Burt and Trivers 2006; van Doorn and Kirkpatrick 2007).

CONCLUSION AND FUTURE DIRECTIONS

The results indicate that female mating biases in *Drosophila* might cause a net decrease to offspring fitness. This is not simply an artifact of the tournament mate-trial setting because mating latency trials independently confirm that females mate more readily with males designated as “preferred” (see Fig. S2). Mating biases that reduce offspring fitness seem paradoxical, yet three factors could potentially explain the persistence of such a pattern of female choice. First, males might provide a direct benefit to their mates, which could potentially counteract any indirect costs (e.g., Oneal et al. 2007). Although current evidence from *D. melanogaster* suggests that direct effects of female preferences are not beneficial and instead could be costly (Friberg and Arnqvist 2003), this scenario would be an interesting topic for future research. A second possibility is that *Drosophila* female mating biases are passive rather than active. For example, male mating success is at least partially a function of locomotor activity, with high-activity males encountering and consequently mating with more females than low-activity males. A passive female mat-

ing bias of this variety is not directly costly to females because there is no energetic cost of searching for a mate (Kokko et al. 2006; Kotiaho and Puurtinen 2007), and is likely to have sexually antagonistic fitness consequences for offspring (Long and Rice 2007). Furthermore, females are only expected to evolve to resist males carrying female-detrimental genes if the cost of active mate choice is less than the indirect costs of having less fit offspring. Finally, multiple mating might mitigate the indirect fitness costs that we observe here, in single mating contexts. We estimated the fitness of offspring from females that were singly mated to a preferred or unpreferred male. In natural contexts, *Drosophila* females mate multiply, which could help to eliminate sexually antagonistic consequences for offspring if females preferentially use X-bearing sperm from “unpreferred” males and Y-bearing sperm from “preferred” males.

The sex ratio bias observed for unpreferred fathers has adaptive consequences for both parents. Unpreferred males and their mates have high-fitness daughters and benefit by producing daughters in excess of sons. By producing offspring with a higher mean fitness, unpreferred fathers might make a greater long-term genetic contribution to the population than might be expected based on their relative mating success. In other words, male mating success variance might be considerably higher than the actual fitness variance among males.

Sexually antagonistic selection was largely ignored experimentally until the last several years, but steadily mounting evidence now indicates that it is an important mechanism maintaining population genetic variation for fitness (e.g., Chippindale et al. 2001; Foerster et al. 2007; Cox and Calsbeek 2009). The potential ubiquity of sexually antagonistic variation, coupled with a variety of sex ratio distortion mechanisms (e.g., Clutton-Brock and Iason 1986; Jaenike 2001), suggests that the results reported here might commonly occur in nature. To our knowledge, only one other such report, in an *Anolis* lizard species (Calsbeek and Bonneaud 2008), has been published. Future work in additional animal species might benefit by integrating female choice assays with analyses of sex ratio modification and sex-specific offspring fitness.

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Supporting Information

The following supporting information is available for this article:

Figure S1. The male mating success trial design includes three rounds of competition during which successful males (right) or unsuccessful males (left) are retained for breeding experiments.

Figure S2. Relationship between different female preference assays.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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