Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females

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Abstract

The evolution of female mate choice, broadly defined to include any female behaviour or morphology which biases matings towards certain male phenotypes, is traditionally thought to result from direct or indirect benefits which females acquire when mating with preferred males. In contrast, new models have shown that female mate choice can be generated by sexual conflict, where preferred males may cause a fitness depression in females. Several studies have shown that female *Drosophila melanogaster* bias matings towards large males. Here, we use male size as a proxy for male attractiveness and test how female fitness is affected by reproducing with large or small males, under two different male densities. Females housed with large males had reduced lifespan and aged at an accelerated rate compared with females housed with small males, and increased male density depressed female fitness further. These fitness differences were due to effects on several different fitness components. Female fitness covaried negatively with male courtship rate, which suggests a cost of courtship. Mating rate increased with male size, whereas female fitness peaked at an intermediate mating rate. Our results suggest that female mate choice in *D. melanogaster* is, at least in part, a by-product of sexual conflict over the mating rate.

Introduction

Female mate choice results from any trait in females (e.g. behaviour, structure or physiology) which biases matings towards certain male phenotypes (e.g. Maynard-Smith, 1987; Kirkpatrick & Ryan, 1991; Andersson, 1994). The adaptive value of female mate choice behaviour remains elusive in most cases (see Andersson, 1994). Mate choice involves costs (e.g. Alatalo *et al.*, 1987; Pomiankowski, 1987; Gibson & Bachman, 1992; Reynolds & Côté, 1995), and it therefore seems reasonable that females must gain appreciable benefits from mating with some males over others. Theoretical research in this field has focused on four scenarios: direct benefit models (e.g. Heywood, 1989; Price *et al.*, 1993; Iwasa & Pomiankowski, 1999), the Fisherian runaway process (e.g. Lande, 1981; Kirkpatrick, 1982; Pomiankowski *et al.*, 1991; Day, 2000), 'good genes' models (e.g. Grafen, 1990; Iwasa *et al.*, 1991; Houle & Kondrashov, 2002; Kokko *et al.*, 2002) and sexual conflict (Holland & Rice, 1998; Gavrilets, 2000; Gavrilets *et al.*, 2001; see also Parker, 1979).

If males provide females with substantial direct benefits which elevate her immediate fecundity and/or fertility, the direct benefits of choice can often be expected to outweigh the costs of choice (Iwasa & Pomiankowski, 1999). It is more difficult to see how such costs can be counterbalanced when females receive little but sperm. Female mating preferences might evolve simply as a result of being genetically correlated with male attractiveness, by a process known as the Fisherian run-away process (Fisher, 1930). In this scenario, females that exercise choice gain indirect genetic benefits by the superior reproductive success of their sons ('sexy sons'). However, this process does not account for the origin of female mate choice (Kirkpatrick, 1987) and fails to

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explain how mate choice can be generally maintained in the face of the costs that may be involved (Pomiankowski, 1987).

Costly female mate choice might instead have originated and been maintained by indirect genetic viability benefits to offspring provided by males ('good genes') (e.g. Andersson, 1986; Pomiankowski, 1987). These models, however, face several problems related to the heritability of fitness. Theory predicts that heritability of fitness should be deflated by selection, and this process should be reinforced if mate choice becomes established thus reducing the potential benefits of mate choice (the 'lek paradox'; Borgia, 1979; Kirkpatrick & Ryan, 1991). However, the gravity of this problem has been much debated. Empirical data suggest that the heritability of fitness in nature is low (Burt, 1995, 2000), and that the indirect viability benefits that could be gained in this way are minor (see Møller & Alatalo, 1999). Several theoretical studies have suggested ways in which significant heritability of fitness could be maintained in the face of selection (e.g. Pomiankowski & Møller, 1995; Rowe & Houle, 1996). It is also difficult to see how mate preferences could bring about major indirect genetic benefits if fitness is determined to a large extent by epistatic gene interactions. While the extent of epistasis for fitness is debated (Whitlock *et al.*, 1995), recent studies of *Drosophila* have revealed strong epistatic interactions for males fitness, due to interactions between loci located on the Y-chromosome and the rest of the genome (Chippindale & Rice, 2001), and a negative genetic correlation of adult fitness between the sexes (Chippindale *et al.*, 2001).

Female mate choice can also result from sexual conflict over mating (Arak & Enquist, 1995; Holland & Rice, 1998; Gavrilets *et al.*, 2001). For example, as male mating rate constitutes a substantial part of male fitness in polyandrous mating systems, sexual selection on male mating rate is generally positive and directional (Arnold & Duvall, 1994). In contrast, females are expected to have an optimal mating rate (Arnqvist & Nilsson, 2000). Matings have both positive and negative impact on female fitness (for reviews, see Arnqvist & Nilsson, 2000; Jennions & Petrie, 2000), but while costs should accumulate over successive matings, the per-mating benefits should decrease. This will result in an evolutionary conflict between the sexes over the mating rate (Parker, 1979; Holland & Rice, 1998; Arnqvist & Nilsson, 2000). Similar conflicts can occur, for example over the timing of mating or the mating environment (Holland & Rice, 1998).

Mutations which increase male mating success are often predicted to spread in a population, even if this comes at a cost to females due to suboptimal mating (Parker, 1979). This will favour increased resistance to mate in females. Female resistance will result in mate choice as it will bias matings towards males which express high levels of 'stimuli'. When genes controlling female mating decisions have pleiotropic effects, females will face tradeoffs (Gavrilets *et al.*, 2001). This is highly likely as the senses involved in mate choice are also used in other contexts, such as food acquisition and predator detection. It has repeatedly been suggested that female mating preferences can arise as incidental by-products of adaptive evolution caused by natural selection (West-Eberhard, 1984; Kirkpatrick, 1987; Ryan, 1998; Noor, 2000). Models of this process (Holland & Rice, 1998; Gavrilets *et al.*, 2001) predict that both sexes will be subjected to opposing natural and sexual selection, and that this antagonistic coevolutionary 'chase' could lead to exaggerated secondary sexual traits in males and costly female mate choice.

Testing the predictions of various models for the evolution of female mate choice empirically has proven to be exceedingly difficult, because predictions regarding male traits are often shared by several, or even all, models (Kirkpatrick, 1987; Kirkpatrick & Ryan, 1991; Andersson, 1994). For example, studies showing a genetic correlation between male traits and female preference have been interpreted as supporting Fisherian scenarios, while studies showing that females mate with males in better condition have been seen as supporting the 'good genes' scenario. Unfortunately, however, all models of the evolution of female mate choice can accommodate observations of positive genetic correlations, due to assortative mating and the resulting linkage disequilibrium (Pomiankowski & Sheridan, 1994). All models also either directly predict, or do so in their extension, that females should mate preferentially with males in better condition. This is because male traits will evolve to be costly in terms of natural selection, and hence be condition-dependent in expression, under all scenarios (Rowe & Houle, 1996). More informative tests of these models should instead focus on selection of female mating preferences (e.g. Kirkpatrick, 1987; Kirkpatrick & Ryan, 1991). For example, models of the evolution of female mate choice by antagonistic coevolution are unique in predicting that preferred males could actually cause a direct fitness depression in females (Gavrilets *et al.*, 2001), in either of two possible ways. First, attractive males could cause females to mate too frequently, by elevating their mating rate (Arnqvist & Nilsson, 2000) or by inducing costly extra-pair matings (Petrie & Kempenaers, 1998). Secondly, attractive males may also confer a higher per-mating cost on females if the preferred trait is positively associated with the cost of mating to females. This will be true if preferred males increase either the ecological costs of mating, such as mating at suboptimal times or locations (Holland & Rice, 1998), or the physiological costs of mating, such as transferring more toxic substances in their ejaculate (Chapman *et al.*, 1995; Rice, 1996, 1998; Civetta & Clark, 2000; Johnstone & Keller, 2000).

Here, we assess various models for the evolution of female mate choice by measuring the fitness effects in females of reproducing with males of varying degree of

attractiveness to females. This allows powerful and unambiguous tests of the effects of particular male phenotypes on female fitness, because we experimentally control both male phenotype and the number of mates. We performed a large laboratory experiment, using *Drosophila melanogaster*, where one group of females was assigned preferred male phenotypes and another assigned less preferred male phenotypes, under two different male densities. The experiments lasted throughout the females' life, and a number of different fitness components were measured while assaying the reproductive behaviour of all individuals.

Materials and methods

Fly stock

We used Dahomey wild-type flies, which were collected in 1970 at Dahomey (now Benin) and has been maintained in mass culture in population cages ever since (for culture protocol, see Partridge & Farquhar, 1983). Five months prior to the experiments, eggs were collected from the Dahomey wild-type stock and reared in bottle cultures in a scheme designed to imitate cage rearing. Eighteen bottles (200 ml) with approximately 150 flies in each were started each week, holding population density fairly constant. These bottles were part of the total stock of flies for 4 weeks, rendering the effective culture size of 72 bottles at any given time. When a new set of 18 bottles where started, flies from bottles of age 2, 3 and 4 weeks were culled and mixed randomly to culture these. New bottles thus contained flies of all ages and bottles, reducing age specific fecundity selection (Sgrò & Partridge, 2000). All flies were fed with standard cornmeal food and maintained at 25 °C at a 12 h : 12 h light : dark cycle in rearing cabinets.

Experimental rationale

It is important to note that our operating definition of female mate choice (e.g. Maynard-Smith, 1987; Kirkpatrick & Ryan, 1991) is very wide and collectively describes a large range of processes causing mating biases among males, from indirect/passive attraction/resistance and various forms of male exploitation of sensory biases in females to more direct/active mate assessment scenarios (see Wiley & Poston, 1996 for a discussion). Male *D. melanogaster* perform a complex courtship (Hall, 1994). Although all components included seem important for female acceptance of any given male, scoring male 'attractiveness' by courtship alone has proven difficult. Nonrandom mating by size in males is extremely common in insects (see reviews by Thornhill & Alcock, 1983; Choe & Crespi, 1997), and *D. melanogaster* is no exception to this rule (Partridge, 1988). It is clear that both male–male competition and female mate choice contributes to nonrandom mating in *D. melanogaster* (Greenacre *et al.*, 1993; Markow, 1988; Iliadi *et al.*, 2001). It is also clear that male size is under selection by mate choice (Ewing, 1961, 1964; Partridge & Farquhar, 1983; Markow, 1986, 1988; Partridge *et al.*, 1987a,b; Wilkinson, 1987; Pitnick, 1991), although it is not clear whether this is the result of some form of active female mate assessment of male size *per se* or whether it is the result of female preferences for male characters which are correlated with male size (e.g. higher persistence in courtship, stronger courtship stimuli) (Partridge, 1988). Irrespective of whether females exhibit a preference for male size *per se* or whether the target of their preference is a trait in males which is correlated with general size, females will bias matings towards larger males. The fitness consequences for females of direct and indirect choice of large males will thus be very similar. In this study, we hence used male size a proxy for male 'attractiveness' to females.

Experimental methods

We generated variation in male body size according to an experimental protocol where larval food quality was varied. We introduced groups of 75 pairs of flies into 200 ml bottles. After 12 days, all adult flies were removed and virgin males and females were collected on day 12 and 13 (early period). The adult flies where then reintroduced into these bottles for another 12 days for continued egg laying. On day 25, they were again removed and virgin males were collected on days 25 to 27 (late period). All virgin flies were collected within 5 h of eclosion and were housed individually in vials (40 mm depth, 14 mm diameter), containing 1.5 ml yeasted food medium, for 3 days until they were used in the experiments described below. Female virginity was checked by inspecting storage vials for larvae. One day after eclosion, the body size of all males was measured. We used thorax length, measured from the midpoint of the anterior margin of the thorax to the distal midpoint of the scutellum, as a measure of body size. Thorax length has proven to be a good measure of general size, as it correlates closely with other measures of body size such as wing length (Robertson & Reeve, 1952). Measurements were taken using a digitizing tablet (Summasketch® III; CalComp Technology, Inc., Anaheim, CA) under a sidemounted camera lucida attached to a dissecting microscope (Leica® MZ8; Leica AG, Heerbrugg, Switzerland).

We used two size classes of males in our experiments. Small males had a thorax length between 0.60 and 0.75 mm and large males between 0.85 and 0.95 mm. Most large males were collected during the early period and most small males derived from the late period (see above). All males included in our experiments were, however, solely chosen by their size, regardless from which period they were collected. All females used in the experiment were collected during the early period. Note

that even if flies derive from different collection periods (see above), all flies were of identical adult age (3 days) at the start of the experiments.

To study the effects of male size on female lifetime fitness, we used a 2 × 2 factorial design with the factors male size (L = large and S = small males, as defined above) and male density (one or two males per female). Our density treatment aimed to simulate different male encounter rates for females and to increase the potential for active female mate assessment. Females ($n = 140$) were randomly assigned treatment combinations (S1, $n = 33$; S2, $n = 36$; L1, $n = 36$; L2, $n = 35$). Each replicate female was housed individually with her male(s) in a vial (100 mm depth, 27 mm diameter) containing 10 ml food medium sprinkled with live yeast. All replicates were transferred to a fresh vial every day, until the death of the female. If a male died before the female, a similar aged male which had experienced similar conditions, replaced him no later than the following day. Female egg production was recorded every 24 h. The number of adult offspring resulting from these eggs was counted on day 17. Fly transfer in these experiments was conducted under light anaesthesia (CO_2). No flies were anaesthetized earlier than 3 h post-eclosion.

To quantify reproductive behaviour, we performed spot-checks of each replicate seven times per day. Spot-checks were separated by an hour or more, and continued throughout each female's life. Any courtship and/or mating observed during spot-checks were recorded. Note that these behavioural assays were not meant to record every single courtship or mating in every single replicate, but rather to provide relative measures of the frequency of these behaviours across replicates.

More detailed assays of female and offspring performance were made on three occasions in each female's life; during days 4–6, 14–16 and 24–26 (fewer if the female died prior to the last period). During these three periods, the number of unhatched eggs was counted 26 h after the female had been transferred to a new vial. These eggs were either unfertilized or nonviable, as eggs of *D. melanogaster* hatch within 24 h at 25 °C (Ashburner, 1989). Larval development time (time from egg to adult) was also measured from these vials, by counting the number of flies that eclosed twice a day (morning and late afternoon). Statistical evaluations were performed with SYSTAT® (SPSS, Chicago, IL, USA).

Results

Effects of male size and density on female fitness

The total number of adult offspring a female produced during her life represents a measure of her fitness, which can be decomposed into three components: survival, fecundity rate and egg-adult offspring survival. The effects of the treatments on fitness and its components are summarized in Table 1. Both male size and male

Table 1 Analyses of variance and covariance of female net fitness and its components.

Response variable	Source	d.f.	MS	*F*	*P*
Lifetime offspring production*	Male size	1	1.20909×10^{13}	9.576	0.002
	Male density	1	8.80059×10^{12}	7.652	0.009
	Male size × male density	1	3.51052×10^{11}	0.278	0.599
	Error	136	1.26258×10^{12}		
	($R^2 = 0.108$)				
Female lifespan	Male size	1	1026.913	8.228	0.005
	Male density	1	1687.422	13.520	<0.001
	Male size × male density	1	129.553	1.038	0.310
	Error	136	124.809		
	($R^2 = 0.141$)				
Female fecundity†	Male size	1	96454.134	0.454	0.501
	Male density	1	27496.458	0.129	0.720
	Male size × male density	1	132.674	0.001	0.980
	Female lifespan	1	3.37068×10^{7}	158.727	<0.001
	Error	135	212357.170		
	($R^2 = 0.577$)				
Proportion of eggs surviving to adulthood‡	Male size	1	0.348	6.854	0.011
	Male density	1	0.006	0.116	0.734
	Male size × male density	1	0.000	0.000	0.992
	Female lifespan	1	2.093	41.248	<0.001
	Error	135	0.051		
	($R^2 = 0.265$)				

*Transformed as $X' = X^2$.
†Measured as the total number of eggs produced.
‡Transformed as $X' = X^4$.

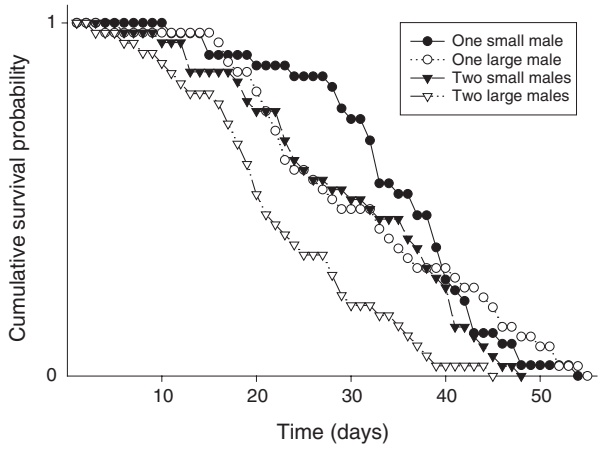

Fig. 1 Cumulative survival probability for females in the four treatment combinations.

density affected female fitness negatively [mean number of adult offspring per female (SE): S1 = 1379 (94); S2 = 1234 (86); L1 = 1214 (80); L2 = 957 (73)]. Females housed with large males produced on average 16% less adult offspring compared with those housed with small males, and females housed with two males produced on average 14% less adult offspring compared with those housed with only one male. Further analyses of each of the fitness components provided insights into the causes of these effects. Female lifespan was significantly reduced both by large males and by two males (Fig. 1), whereas female fecundity rate, defined as the total number of eggs laid controlled for female lifespan (as females living longer produced more eggs), was not significantly affected by either of our treatment factors. We defined egg-adult survival as the proportion of eggs laid by a given female which produced adult flies, while controlling for female lifespan as hatchability of eggs and larval survival decreases with female age in *D. melanogaster* (Kern *et al.*, 2001). Egg-adult survival was lower in females housed with large males compared with those housed with small [large males mean (SE) = 0.77 (0.02); small males mean = 0.82 (0.02)] but was not significantly affected by male density. Our results thus show that *D. melanogaster* females suffer a reduction in fitness when housed with large males and with two males, and that these effects were mediated through a reduced lifespan of females and a lower age-specific egg-adult survival of their offspring.

Effects of male size and density on mating and courtship rates

Our analyses of the effects of male size and density on courtship and mating rates (Table 2) were restricted to rates measured during the first 7 days of each female's life. This is because 75% of the observed matings took place within this time and measures of mating and courtship rates based on the entire lifetime thus would be confounded by variation in lifespan. As previous studies have shown that courtship and remating increases with male size (Partridge *et al.*, 1987a,b; Pitnick, 1991) and with sex ratio (Dernoncourt-Sterpin *et al.*, 1991; Chapman & Partridge, 1996) in *D. melanogaster*, we used one-tailed tests when evaluating the *a priori* alternate hypotheses that females housed with large males (two males) were courted and mated more frequently than females housed with small males (one male). Effects of

Table 2 Analyses of variance of the effects of male size and density on courtship and mating rates.

Response variable	Source	d.f.	MS	*F*	*P*‡
Courtship rate*	Male size	1	1.154	12.730	<0.001
	Male density	1	0.586	6.462	0.006
	Male size × male density	1	0.010	0.108	0.743
	Error	136	0.675		
	($R^2 = 0.102$)				
Mating rate*	Male size	1	0.101	3.726	0.027
	Male density	1	0.020	0.721	0.199
	Male size × male density	1	0.037	1.367	0.244
	Error	136	0.027		
	($R^2 = 0.042$)				
Remating†	Male size	1	0.513	2.772	0.050
	Male density	1	0.176	0.950	0.167
	Male size × male density	1	0.425	2.296	0.134
	Error	73	0.185		
	($R^2 = 0.076$)				

*Transformed as $X' = X^{1/2}$, N = 140.
†Transformed as $X' = X^{1/5}$, N = 77. Analysis includes only females that were observed to remate.
‡Test involving the main effects of size and density report one-tailed *P*-values (see text).

the interactions are less obvious, and these were thus tested with two-tailed tests. The effects of male size were in agreement with earlier studies: large males performed a higher courtship rate [mean values (SE) of groups: S1 = 1.51 (0.12), S2 = 1.92 (0.16), L1 = 2.03 (0.14), L2 = 2.34 (0.14)] and females housed with large males mated more frequently [S1 = 0.229 (0.02), S2 = 0.178 (0.02), L1 = 0.250 (0.03), L2 = 0.244 (0.02)]. The interpretation of the latter analysis should, however, be considered with some caution, as we were unable to fulfil the assumption made in general linear models of normality within groups (Zar, 1996). Females also received a higher courtship rate when housed with two males, but no significant difference in mating rate could be attributed to male density. To further dissect female remating behaviour, we tested for differences in time between first and second mating (Pitnick, 1991), using the subset ($n = 77$) of females that was observed to remate. This analysis gave results very similar to those of mating rate (Table 2).

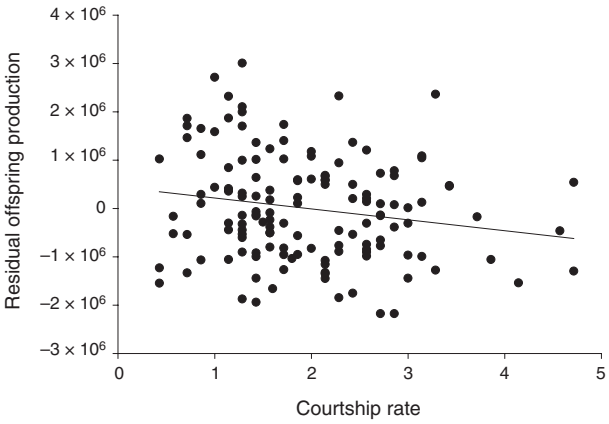

Fig. 2 Female lifetime offspring production in relation to the courtship rate to which they were exposed. Offspring production represents residuals from an analysis of covariance including male size, male density, their interaction, mating rate and the quadratic term of mating rate.

Covariation between female fitness and mating and courtship rates

Male size and density *per se* do not offer any mechanistic explanations for the reduction of female fitness. Earlier studies have shown that especially mating (Fowler & Partridge, 1989; Chapman *et al.*, 1995) but possibly also courtship (Partridge & Fowler, 1990; Holland & Rice, 1999) reduces female fitness in *D. melanogaster*. While increased exposure to courtship should reduce female fitness monotonically, female fitness should be maximized at some intermediate mating rate where females balance the need for viable sperm while avoiding the costs of excessive mating (see Arnqvist & Nilsson, 2000). Thus, we evaluated the effects of observed mating and courtship rates on female fitness by simultaneously adding the first-order term for courtship rate and the first- and second-order terms for mating rate as covariates to the model of female fitness presented in Table 1. This procedure allows female fitness to be linearly related to courtship rate and nonlinearly related to mating rate. When reanalyzing female fitness (model 1 in Table 1) using these covariates, courtship rate was indeed negatively correlated with female fitness (Fig. 2, Table 3). Further, the second-order term of mating rate was significant while the first-order was not (Table 3), suggesting an optimal observed mating rate which was not significantly different from zero (Fig. 3). Note, however, that our estimate of mating rate measures the least possible number of matings a given female performed, and that the position of the true optimum is most likely higher than this. The effect of courtship rate removed some of the variance attributed to male size and density when included in the model, but did not leave these factors nonsignificant, implying that they have effects beyond being associated with mating and courtship rates.

The analysis of covariance was tested for interactions among factors and covariates. None of these interactions were significant and a test showed that the model was not significantly improved by collectively adding the interaction terms (multiple partial F-test; $F_{6,127} = 0.57$, $P = 0.38$).

Courtship and mating rates were also added as covariates to each of the analyses of female fitness components. When analyzing female lifespan the interactions between covariates and factors collectively improved model fit ($F_{6,127} = 2.23$, $P < 0.05$). A closer analysis of this effect revealed that while the interactions between courtship rate and the main factors strongly improved the model ($F_{2,131} = 5.318$, $P < 0.01$), the interactions between mating rate and the main factors did not ($F_{4,129} = 1.081$, $P = 0.184$). The model including the three covariates and the interactions with courtship rate is presented in Table 3. The effect of male size on female lifespan seen in the initial analysis of variance (Table 1) was to a large extent accounted for by our behavioural variables and the interactions with courtship rate. Females exposed to large males experienced a higher courtship rate than did those exposed to small males, and courtship rate appeared to be negatively related to female lifespan in the former, but not in the latter group of females. The negative effects of male density on female lifespan remained after controlling for behaviour (Table 3). Male density also interacted with courtship rate. Intriguingly however, while courtship rate was negatively related to female lifespan under low male density this appeared not to be the case in the high male density treatment. The relationship between mating rate and female lifespan was, however, nonlinear.

When analyzing female fecundity rate, none of the interactions between the four covariates and factors were

Table 3 Analyses of covariance of female fitness and its components.

Response variable	Source	d.f.	MS	*F*	*P*
Lifetime offspring production*	Male size	1	6.05983×10^{12}	5.061	0.026
	Male density	1	6.67011×10^{12}	5.571	0.020
	Male size × male density	1	7.54590×10^{11}	0.630	0.429
	Mating rate	1	3.90009×10^{12}	3.257	0.073
	Mating rate × mating rate	1	6.07558×10^{12}	5.074	0.026
	Courtship rate	1	6.29600×10^{12}	5.242	0.024
	Error	133	1.19729×10^{12}		
	($R^2 = 0.173$)				
Female lifespan	Male size	1	45.234	0.390	0.534
	Male density	1	1637.200	14.101	<0.001
	Male size × male density	1	290.449	2.502	0.116
	Mating rate	1	440.916	3.797	0.053
	Mating rate × mating rate	1	587.297	5.058	0.026
	Courtship rate	1	379.113	3.265	0.073
	Male size × courtship rate	1	316.136	2.723	0.101
	Male density × courtship rate	1	746.328	6.428	0.012
	Error	131	116.109		
	($R^2 = 0.231$)				
Female fecundity†	Male size	1	328473.391	1.606	0.207
	Male density	1	19.320	0.000	0.992
	Male size × male density	1	12771.716	0.062	0.803
	Female lifespan	1	3.05814×10^{7}	149.563	<0.001
	Courtship rate	1	1455184.489	7.117	0.009
	Mating rate	1	161006.896	0.787	0.376
	Mating rate × mating rate	1	188621.422	0.922	0.339
	Error	132	204472.042		
	($R^2 = 0.602$)				
Proportion of eggs surviving to adulthood‡	Male size	1	0.314	6.133	0.015
	Male density	1	0.002	0.039	0.843
	Male size × male density	1	0.001	0.010	0.921
	Female lifespan	1	2.027	39.581	<0.001
	Courtship rate	1	0.010	0.198	0.657
	Mating rate	1	0.022	0.435	0.511
	Error	133	0.051		
	($R^2 = 0.271$)				

*Transformed as $X' = X^2$.
†Measured as the total number of eggs produced.
‡Transformed as $X' = X^4$.

significant and they did not collectively improve model fit ($F_{8,124} = 1.640$, $P = 0.060$) (Table 3). While male size and density had no apparent effect on female fecundity rate (Table 1) the addition of the covariates mating rate and courtship rate indicated that high courtship rate reduces female fecundity rate (Fig. 4). Egg-adult offspring survival was also analysed in much the same way. However, we did not include the second-order term of mating rate here. This is because mating rate should primarily, if at all, affect offspring performance via maternal effects. Offspring survival could thus be a monotonically decreasing function of mating rate in *D. melanogaster* as elevated mating rates could have negative impacts upon the general performance of females (see introduction). The interactions between the three covariates and factors were again all nonsignificant and did not collectively improve model fit ($F_{6,127} = 0.85$, $P = 0.27$). We found no relationship between courtship or mating rates and our measure of offspring survival (Table 3). The effect of male size found in the initial model (Table 1) was still present after controlling for variation in mating rate and courtship rate, indicating that the effects of male size on offspring survival is unrelated to differences in behaviour.

Offspring performance

Our assays of pre-adult offspring performance allowed us to partition the effect of male size on egg-adult offspring survival into effects on egg hatchability and larval survival. As none of the behavioural variables affected egg-adult offspring survival, they were excluded from

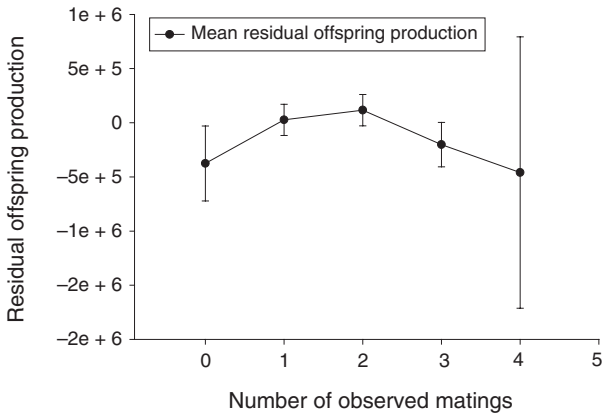

Fig. 3 Female fitness in relation to the observed number of matings during the first 7 days of our experiment. Figure shows residual lifetime offspring production, generated in analysis of covariances including male size, male density, their interaction and courtship rate. Error bars represent SE.

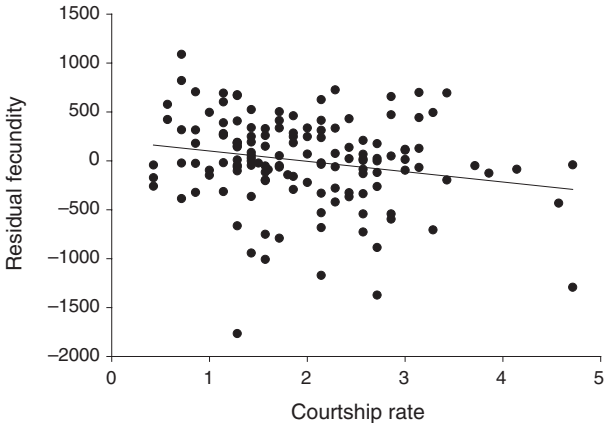

Fig. 4 Female fecundity rate in relation to the courtship rate to which they were exposed. Offspring production represents residuals from an analysis of covariance including male size, male density, their interaction, female lifespan, mating rate and the quadratic term of mating rate.

these analyses. We first restricted our analyses to offspring produced early in a female's life (the first assay: days 4–6 in each female's life). These analyses failed to reveal any effects of male size or density on either egg hatching rate or larval survival (Table 4). These analyses are statistically relatively powerful, as most of the females survived to this age, but do not capture effects expressed later in life. We therefore performed a series of repeated measures analyses of variance. These analyses included only females that survived 26 days or more, and mean values for days 4–6, 14–16 and 24–26 made up the three repeated measures over time. These analyses (Table 5) showed that both egg hatchability and larval survival declined over time. They also showed that both measures of offspring performance decreased more rapidly in females housed with large males than in females housed with small males (Fig. 5), as revealed by the interaction terms between male size and time (Table 5). Male size also had a significant effect, taken over all three periods, on egg hatchability but not on larval survival. In summary, the effect of male size on egg-adult offspring survival seen in the initial analyses is explained by a more rapid decrease in both egg hatchability and larval survival among females housed with large males compared with those housed with small males.

Our third independent measure of offspring performance, offspring development time, was analysed in much the same way as egg hatchability and larval survival. Development time was measured as the time until half of the offspring that eventually enclosed from a given vial had done so. Development time was adjusted by using the residuals from a quadratic model fitting number of hatched eggs to development time, to compensate for differences in development time caused by density-dependent growth rate. The analysis of development time in offspring produced early (days 4–6) showed that offspring from females housed with large males developed slower than offspring from females housed with small males (mean difference in days 0.34, SE = 0.07) (Table 4).

In contrast to hatching rate and larval survival, no previous analysis has explored the covariance between development time of offspring and the mating and courtship behaviours of their parents. For reasons detailed above, we did not include the second-order term of mating rate. Both mating rate and courtship rate were highly significant (Table 4) and both prolonged development time when high, and these two behavioural variables explained much of the effect of male size on offspring development time. A full model with the interactions between covariates and factors was also performed. The addition of the interaction terms did not improve model fit ($F_{4,125} = 0.13$, $P = 0.486$).

A repeated measures analysis of variance including only the subset of females that survived through all three time periods (see above), did not show any significant temporal trends in offspring development time (Table 5).

Covariation between behaviour and offspring production early in life

The covariance between our behavioural variables and female fitness described above were based on courtship and mating rates experienced early in life by females. To assess more immediate relationships between the behavioural variables and offspring production, we restricted our analysis to include only female offspring production during the first 7 days. This analysis (Table 6) showed that increasing courtship rate was strongly associated with decreased offspring production and that offspring

Table 4 Analyses of variance and covariance of different components of pre-adult offspring fitness, for offspring produced by females during days 4–6 of the experiment.

Response variable	Source	d.f.	MS	*F*	*P*
Egg hatching rate*	Male size	1	0.003	0.180	0.672
	Male density	1	0.015	0.943	0.333
	Male size × male density	1	0.000	0.000	0.993
	Error	133	0.016		
	($R^2 = 0.008$)				
Larval survival†	Male size	1	0.002	0.194	0.660
	Male density	1	0.000	0.029	0.864
	Male size × male density	1	0.003	0.329	0.567
	Error	132	0.009		
	($R^2 = 0.004$)				
Development time‡	Male size	1	3.858	9.829	0.002
	Male density	1	0.248	0.632	0.428
	Male size × male density	1	0.171	0.435	0.511
	Error	131	0.393		
	($R^2 = 0.073$)				
Development time‡	Male size	1	0.914	2.609	0.109
	Male density	1	0.034	0.096	0.757
	Male size × male density	1	0.393	1.123	0.291
	Mating rate	1	2.933	8.374	0.004
	Courtship rate	1	4.434	12.661	0.001
	Error	129	0.350		
	($R^2 = 0.188$)				

*Transformed as $X' = \mathrm{Arcsin}(X)$.
†Transformed as $X' = [\mathrm{Arcsin}(X)]^2$.
‡Residuals from a quadratic regression model of development time on larval density.

production peaked at an intermediate mating rate different from zero. Adding the interactions between factors and covariates did not improve model fit ($F_{6,127} = 1.497$, $P = 0.092$).

Discussion

Male size and density had a range of independent effects on several components of female fitness. Females housed with large males had a lower fitness than did females housed with small males, and this effect was primarily due to a reduction in lifespan and to an accelerated rate of decrease in egg-adult offspring survival over time. High male density also depressed female fitness by shortening female lifespan but this effect did not interact with that of male size. Several independent lines of evidence also suggest that high courtship rates are detrimental to females, and that female fitness appears to peak at an intermediate mating rate. Although it is not clear whether male size *per se* is the target of female choice in *D. melanogaster*, our results are in line with previous findings showing that females bias matings towards large males (Ewing, 1961, 1964; Partridge & Farquhar, 1983; Partridge *et al.*, 1987a; Markow, 1986; Pitnick, 1991): females housed with large males indeed remated more rapidly and exhibited higher overall mating rates. How our results agree with the assumptions and predictions of various models for the evolution of female mate choice have been discussed below.

Direct benefits

Models of female mate choice based on direct benefits to females (Heywood, 1989; Price *et al.*, 1993; Iwasa & Pomiankowski, 1999) predict that female fitness should be elevated when females are mated with preferred males as these provide females with direct resources. In several species of *Drosophila* males have been suggested to transfer 'nutrients' to females with their ejaculate (Pitnick *et al.*, 1997) and female mate choice could be maintained if preferred males transfer superior ejaculate donations (Wedell & Sandberg, 1995). If this was the case, these direct benefits should be manifested by an increased fecundity rate, an elevated egg-adult survivorship (i.e. via maternal effects) and/or a prolonged lifespan in females mated with large males. Our results are in direct opposition with these key predictions; we found no effect of male size on fecundity rate but obvious negative effects on both egg-adult survivorship and female lifespan. Moreover, these effects were translated into a negative net effect of male size on female fitness. It is worth noting here that earlier experiments have demonstrated genetic variation among males in 'toxicity' to female *D. melanogaster* (Civetta & Clark, 2000; Sawby & Hughes, 2001).

Table 5 Repeated measures analyses of variance of pre-adult offspring fitness components.

Response variable	Source	d.f.	MS	*F*	*P*
Egg hatching rate*					
Between subjects	Male size	1	1.292	6.7	0.011
	Male density	1	0.110	0.577	0.450
	Male size × male density	1	0.511	2.684	0.106
	Error	74	0.190		
Within subjects	Period	2	9.284	102.344	<0.001
	Period × male size	2	0.499	5.498	0.005
	Period × male density	2	0.090	0.996	0.372
	Period × male size × male density	2	0.302	3.335	0.038
	Error	148	0.091		
Larval survival†					
Between subjects	Male size	1	0.871	1.616	0.208
	Male density	1	1.412	2.618	0.110
	Male size × male density	1	0.040	0.073	0.787
	Error	75	0.539		
Within subjects	Period	2	6.562	18.796	<0.001
	Period × male size	2	2.002	5.735	0.004
	Period × density	2	0.850	2.435	0.091
	Period × male size × male density	2	0.318	0.909	0.405
	Error	150	0.349		
Development time‡					
Between subjects	Male size	1	0.285	0.842	0.363
	Male density	1	0.351	1.038	0.312
	Male size × male density	1	1.260	3.724	0.058
	Error	61	0.338		
Within subjects	Period	2	0.192	0.852	0.429
	Period × male size	2	0.011	0.051	0.951
	Period × male density	2	0.101	0.449	0.639
	Period × male size × male density	2	0.008	0.034	0.966
	Error	122	0.225		

*Transformed as $X' = \text{Arcsin}(X)$.
†Transformed as $X' = [\text{Arcsin}(X)]^2$.
‡Residuals from a quadratic regression model of development time on larval density.

Similarly, Pitnick (1991) found that female *D. melanogaster* produced more eggs when mated with small males and several studies have shown that male ejaculate 'donations' are actually detrimental for females (Fowler & Partridge, 1989; Chapman *et al.*, 1995; Civetta & Clark, 2000). Our results are also concordant with those of a similar, independent and simultaneous study (Pitnick & García-González, 2002) who found that not only female longevity but also fecundity rate decreased when exposed to large males.

Indirect benefits

The expected strength of indirect genetic benefits to offspring will be related to the proportion of phenotypic variance in male attractiveness which is due to genetic variance. In particular, when males reared under identical laboratory conditions are used, phenotypic variance will reflect genetic variance to a maximal extent. This may tend to overestimate true 'good genes' effects, as it will not accurately reflect the conditions in nature where environmental variance plays a more important role. In our experiments, we used a protocol similar to that used under normal culture of this stock, which is aimed at mimicking natural conditions where food quality and crowding varies (see also Partridge & Farquhar, 1983). While this protocol could be seen as conservative in terms of detecting 'good genes' effects (but see Weigensberg & Roff, 1996), as it increases phenotypic variance in body size by elevating environmental variance, it will more accurately reflect conditions under normal culturing and in nature where larval food quality varies (for field data on several *Drosophila* species, see Grimaldi & Jaenike, 1984).

Models based on indirect genetic benefits to offspring for the evolution of female mate choice rely on either increased viability ('good genes') or reproductive success of offspring (see 'Introduction' for references). Under 'good genes' scenarios, female fitness should be elevated when mated with preferred males as a result of increased

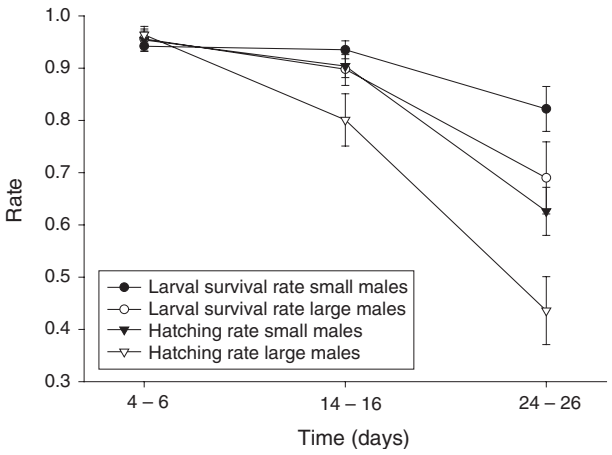

Fig. 5 The average hatching rate of eggs and the larval survival rate (i.e. the proportion of larvae surviving to adulthood) in offspring produced by females housed with small or large males at three different time periods during the experiment. Error bars represent 1SE.

offspring viability expressed under the juvenile and/or adult stages. This scenario is notoriously difficult to reject, because such viability benefits could conceivably be expressed at any stage in life and could take many forms (i.e. survival, competitive ability, fecundity of daughters, etc.) (Jennions & Petrie, 2000; Kokko *et al.*, 2002). It could, however, be argued that general viability benefits should be genetically correlated across multiple offspring fitness components, especially if such components are condition-dependent (Rowe & Houle, 1996).

Thus, although we did not measure adult fitness of offspring in the current study, our measures of offspring viability in the juvenile stages could at least be indicative of more general viability effects. However, the effects of male size on offspring performance were all negative. Offspring of large males had a lower egg hatchability, and if anything a lower larval survival and a slower development time. It is also worth noting that putative 'good genes' effects discussed earlier in *D. melanogaster* (Partridge, 1980; Taylor *et al.*, 1987) should have been detected in our experiments, as they involve fitness effects expressed during the larval stage.

Apart from experimentally varying male attractiveness, our experiments also essentially involved a 'choice' vs. 'no choice' treatment in the form of male density (two vs. one male). Should indirect benefits be crucial to females, one would expect that offspring fitness to be elevated in the high male density treatment after controlling for direct effects of mating and courtship rates. This was, however, clearly not the case (see bottom of Tables 3 and 4).

More importantly, we found that the total offspring production of females mated with large males was 16% lower than that of females mated with small males. In order for indirect genetic benefits to more than outweigh these direct costs to females from mating with large males, and hence result in a net benefit, the average adult fitness of offspring must be at least some 20% higher among offspring fathered by large males. Available empirical data suggest that potential 'good genes' benefits are much lower than this, both in *D. melanogaster* [Partridge, 1980 (2–4.5%); but see Schaeffer *et al.*, 1984] and in general [see Møller & Alatalo, 1999 (1.5%)]. Thus, our results do not support predictions of 'good genes' models for the evolution of female mate choice.

As we did not measure the reproductive success of offspring, we cannot directly evaluate potential 'sexy sons' effects. However, at least three aspects of our results are incompatible with the assumptions and predictions of models based on the Fisherian runaway process (see 'Introduction'). First, the direct costs of mating with preferred male phenotypes were large, and it does not seem likely that genetic 'sexy sons' benefits could more than outweigh these (see Møller & Alatalo, 1999). Secondly, general models of this scenario are unable to

Table 6 Analyses of variance and covariance of female offspring production during the first 7 days of the experiment.

Response variable	Source	d.f.	MS	*F*	*P*
Offspring production*	Male size	1	2.37465×10^{9}	0.203	0.653
	Male density	1	1.27830×10^{8}	0.011	0.917
	Male size × male density	1	1.60871×10^{8}	0.014	0.907
	Error	136	1.16830×10^{10}		
	($R^2 = 0.108$)				
Offspring production*	Male size	1	1.97751×10^{10}	1.912	0.169
	Male density	1	3.02636×10^{8}	0.029	0.864
	Male size × male density	1	7.72918×10^{8}	0.075	0.785
	Courtship rate	1	8.61490×10^{10}	8.331	0.005
	Mating rate	1	8.55364×10^{10}	8.272	0.005
	Mating rate × mating rate	1	1.29485×10^{11}	12.521	0.001
	Error	133	1.03410×10^{10}		
	($R^2 = 0.136$)				

*Transformed as $X' = X^2$.

accommodate costly female mate choice (Pomiankowski, 1987), and sizable costs of mate choice seem more likely in *D. melanogaster*. Mate choice involves, by necessity, mate rejection, and several lines of evidence suggest that male harassment and rejection of courting males is costly to *D. melanogaster* females (Partridge & Fowler, 1990; Holland & Rice, 1999). Thirdly, and perhaps most importantly, strict Fisherian models assume that the survival and fecundity of females is unrelated to mate phenotypes (e.g. Kirkpatrick, 1982), and hence that there is no direct selection on female mating preferences (Kirkpatrick & Barton, 1997). This was not the case in our experiments.

There are reasons to believe that attractiveness in males may in fact be negatively genetically correlated with certain offspring fitness components (Brooks, 2000; Chippindale *et al.*, 2001). We have no direct evidence for such genetic effects. Egg-adult survival was lower among females mated with large males, but this effect was due to a more rapid decline in egg-adult survival over time in those females and does probably reflect maternal effects (i.e. ageing; see Fig. 5 and below). One might claim that the prolongation of offspring development time observed among females mated with large males represents a genetic effect, but the fact that this effect was largely accounted for by courtship and mating rates indicates that it is also due to maternal effects (Table 4).

Sexually antagonistic coevolution

Models of female choice generated by sexual conflict of interests in mating (Arnqvist, 1992; Arak & Enquist, 1995; Holland & Rice, 1998; Gavrilets *et al.*, 2001) state that mating preferences evolve as an indirect result of females evolving to reduce/resist direct costs related to mating. Attractive males should be more able to overcome female resistance, and females should hence suffer a fitness depression when exposed to attractive males (Gavrilets *et al.*, 2001). Such fitness depression could, furthermore, be a result of suboptimal mating (rate of mating, timing of mating, place for mating, etc.). It is important to note that these models do not necessarily assume that matings with attractive males should be more costly *per se*, but merely that females should be more likely to mate with males carrying more elaborate secondary sexual traits. Our results show that females housed with attractive males suffer a striking decrease in fitness compared with female housed with less attractive males. Our analyses provided three specific insights into the causes of this effect. First, large males courted females more frequently. Earlier studies have indicated that courtship might be costly to female *D. melanogaster* (Partridge & Fowler, 1990; Holland & Rice, 1999). Our experiments consistently revealed a negative covariation between courtship rate and female fitness components, after controlling for both male size and density as well as for mating rate, and thus strongly suggest that male courtship is costly *per se* to females. Secondly, females mated more frequently when housed with large males. Matings involve a series of costs to female fruitflies (Chapman *et al.*, 1995; Prout & Clark, 2000), and general theory suggests that females should thus exhibit an optimal mating rate representing trade-offs between the various costs and benefits involved (Arnqvist & Nilsson, 2000). Direct empirical support for such optimal mating rates is, however, relatively weak (Mbata *et al.*, 1997; Wilson *et al.*, 1999; Arnqvist & Nilsson, 2000). Our analyses showed that the covariation between observed mating rate and female fitness is nonlinear and shows a maximum at intermediate mating rates (Fig. 3). This effect was apparently not only due to a reduction in lifespan among frequently mating females (Table 3; cf. Fowler & Partridge, 1989; Chapman *et al.*, 1994) but also to a reduction in offspring production rate (Table 6). Collectively, our results are at least in agreement with a scenario where females exhibit optimal mating rates and where large males are more able to 'seduce' females into mating at a suboptimal rate.

Thirdly, egg hatching rate and larval survival decrease with female age in *D. melanogaster*, as a part of a general ageing complex (Kern *et al.*, 2001). Our experiments demonstrated that females housed with large males aged at an accelerated rate compared with those housed with small males. This was obvious both when inspecting offspring performance over time (Fig. 5) and female lifespan. This could be the combined result of the elevated courtship and mating rates. However, while offspring performance decreased more rapidly in females housed with large males, this was not true for females housed with two, rather than one, male(s) (Table 5). Male density affected courtship rate, but not mating rate, in a manner similar to male size (Table 2), suggesting that increased mating rather than courtship rate could be the primary cause of the accelerated rate of ageing observed. In summary, our experiments gave considerable support to models of female mate choice generated by sexually antagonistic coevolution (Holland & Rice, 1998; Gavrilets *et al.*, 2001).

Conclusions

Distinguishing between models of the evolution of female mate choice is notoriously difficult (Kirkpatrick & Ryan, 1991). We have chosen a somewhat different experimental approach, focusing on fitness effects in females of mating with males of varying degrees of attractiveness. Our data show that females suffer direct fitness costs by being exposed to large males, and thus contradict scenarios based on direct benefits. We note that these direct costs became more pronounced over time, and it is therefore not clear exactly how severe they are in natural populations. Further, we found no evidence for indirect genetic benefits, and suggest that

indirect selection on female mating preferences is unlikely to be responsible for female mate choice in *D. melanogaster*. Our results are in line with other recent studies, and suggest that females resists matings because superfluous matings incur direct fitness costs to females. Male size has been shown to relate positively to most components of male courtship in *D. melanogaster*, such as intensity of courtship behaviour and the rate and intensity of courtship song (e.g. Partridge *et al.*, 1987b). We thus suggest that female mate choice for large males in *D. melanogaster* results primarily from sexually antagonistic coevolution, where female preference for large males is due to direct selection on females to generally resist males.

Finally, we wish to stress that the various types of selection pressures acting on female mating preferences discussed above need not be mutually exclusive. For example, given that female resistance and male traits have coevolved to some equilibrium where both traits are costly in terms of natural selection (Gavrilets *et al.*, 2001), we might expect male traits to be condition-dependent in their expression (Rowe & Houle, 1996). Moreover, female resistance and male persistence is predicted to be genetically correlated (Gavrilets *et al.*, 2001). Direct selection for female resistance generated by antagonistic adaptations in males will thus likely be reinforced by indirect selection on female mating biases. However, theoretical analyses suggest that when direct and indirect selection act simultaneously on female mating preferences, the former should overwhelm the effects of the latter (Kirkpatrick, 1996; Kirkpatrick & Barton, 1997).

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