Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*

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Theory predicts that males will benefit when they bias their mating effort towards females of higher reproductive potential, and that this discrimination will increase as males become more resource limited. We conducted a series of experiments to test these predictions in a laboratory population of the fruitfly, *Drosophila melanogaster*. In this species, courtship and copulation have significant costs to males, and females vary greatly in fecundity, which is positively associated with body size. When given a simultaneous choice between small and large virgin females, males preferentially mated with larger, more fecund, females. Moreover, after males had recently mated they showed a stronger preference for larger females. These results suggest that male *D. melanogaster* adaptively allocate their mating effort in response to variation in female quality and provide some of the first support for the theoretical prediction that male stringency in mate choice increases as resources become more limiting.

Keywords: male mate choice; mating costs; *Drosophila melanogaster*; fecundity

1. INTRODUCTION

Traditional models of sexual selection predict that in most animal species, males will be less discriminating in their choice of mating partners than females, because their investment in offspring is much lower (Bateman 1948; Trivers 1972). However, it is becoming increasingly apparent that in many species males, nevertheless, have a high cost of reproduction (mating) due to costs arising from factors such as energetically expensive courtship displays (Judge & Brooks 2001) and the production of ejaculates (Dewsbury 1982; Galvani & Johnstone 1998). If the cost of mating is low for males, in the currency of time lost and/or resources consumed, then low levels of male mate discrimination are predicted (Engqvist & Sauer 2001; Kokko & Johnstone 2002). However, greater mate discrimination by males is predicted when investment into a current mating substantially reduces their future mating opportunities (Partridge & Farquhar 1981; Dewsbury 1982; Simmons 1990; Clutton-Brock & Langley 1997; Bonduriansky 2001). Based on this tradeoff, theory predicts that males of species with higher costs of reproduction will more strongly discriminate among potential mates and more strongly bias their mating toward females that will provide the highest reproductive returns (Parker 1983; Gwynne 1991, 1993; Johnson & Burley 1997; Reinhold *et al.* 2002). Furthermore, it is predicted that with additional matings males should become more stringent in their preference for females of high reproductive value, because the progressive depletion of resources will further increase costs of suboptimal mating (Bonduriansky 2001; Engqvist & Sauer 2001; Kokko & Monaghan 2001).

The most obvious trait influencing the reproductive value of a female is her fecundity (Bonduriansky 2001).

When mating opportunities are constrained, males that show a preference for more fecund females will benefit directly by increasing the number of offspring they produce (Katvala & Kaitala 2001). A growing number of studies investigating male resource allocation under conditions of high mating effort have found that males show a preference for larger, more fecund females (e.g. Gage & Barnard 1996; Gage 1998; Sauer *et al.* 1998; Parker *et al.* 1999; Wedell & Cook 1999; Engqvist & Sauer 2001). Surprisingly, however, there have been few attempts to empirically evaluate whether male mate preferences are sensitive to fluctuating costs of choosing (e.g. scorpionflies, Engqvist & Sauer 2001; fish, Wong & Jennions 2003). Furthermore, we are unaware of any attempt to directly test the prediction that as mating resources become progressively depleted males become more discriminating in mate choice.

The fruitfly *Drosophila melanogaster* provides unique opportunities to test the predictions of male mate choice models. *Drosophila melanogaster* has played an integral role in the development of sexual-selection theory and a great deal is known about the patterns and fitness consequences of female mate choice (Spieth 1952; Partridge 1980; Fowler & Partridge 1989; Chapman *et al.* 1993; Gromko & Markow 1993). Moreover, recent behavioural research has revealed that male *D. melanogaster* vary greatly in their level of interest in females, providing evidence that males have also evolved to mate selectively (Gowaty *et al.* 2003). Several aspects of the reproductive biology of *D. melanogaster* make it a particularly useful species with which to investigate whether male mate choice is influenced by female quality and the costs of choosing (level of resource depletion). First, sexual activity reduces the lifespan of males (Partridge & Farquhar 1981) due to costs arising from vigorous courtship (Cordts & Partridge 1996), the production of ejaculates (Lefevre & Johnson

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1962; Gromko *et al.* 1984) and possibly also due to immunosuppression (McKean & Nunney 2001). Second, repeated mating by males within a 24 h period depletes limiting components of the ejaculate, as indicated by a reduction in the number of offspring per mated female produced after three successive matings (Demerec & Kaufmann 1941; Lefevre & Johnson 1962). Third, the quality of potential female mates is highly variable. This variation largely arises because (i) females vary in their mating history and, therefore, represent different risks of sperm competition and (ii) fluctuations in larval food availability can generate large variance in adult female body size, which is known to be positively associated with fecundity (Lefranc & Bundgaard 2001).

The aims of this study were to (i) establish whether male *D. melanogaster* prefer to copulate with larger, more fecund females and (ii) determine whether male mating effort becomes more biased toward larger females after repeated copulation depletes resources available for insemination.

2. MATERIAL AND METHODS

(a) Culture stocks

Flies were obtained from a large ($n > 1700$ breeding adults per generation) outbred laboratory population (Larry Harshman/moderate density LH_M) that has adapted to the laboratory environment for over 300 generations (see Rice *et al.* 2005). Culture conditions were 12 h light : 12 h dark cycle at 25 °C. Larvae were reared at 150–200 individuals per 10 dram vial provisioned with 10 ml of cornmeal/molasses medium, seeded with approximately 10 mg of live yeast.

(b) Culture of males of standard body size

To obtain males for choice trials, 11 subcultures of the LH_M base population were established by placing 30 pairs of flies (taken directly from the main culture stock) into plastic half-pint containers. Attached to the opening of each inverted half-pint was a circular 'laying plate' that contained standard medium inoculated with a suspension of live yeast. Females were oviposited on the 'laying plates' for 18 h. After this time, 150 eggs were randomly sampled from each plate and placed in a 10 dram culture vial containing 10 ml of culture medium. Vials were incubated under standard conditions (see §2a). On the night before experimentation, we collected males ($97 \pm 2.5\%$ (mean $\pm$ s.d.) of males are at least one day post-eclosion at this time; W. R. Rice & A. D. Stewart 2005, unpublished data) under light CO_2 anaesthesia and stored them at a density of 10 individuals per culture vial. Based on a subsample of experimental flies, the average body mass (dry weight) $\pm$ s.e. of experimental males was 0.248 ± 0.0024 mg ($n = 300$).

(c) Culture of females of small and large body size

To generate females of small and large body size, we manipulated larval density. To obtain eggs, we established 11 subcultures of the LH_M base population (see §2b for subculture procedure). To generate females of small body size, 100 eggs were randomly sampled from an individual laying plate and placed in a 10 dram culture vial ($n = 40$ vials) containing only 1 ml of cornmeal/molasses medium. To generate females of large body size, 50 randomly sampled eggs were removed from an individual laying plate and placed in a 10 dram culture vial ($n = 40$ vials) containing 10 ml of

food. Vials were incubated under standard conditions (see §2a) and after 8 days females were collected under light CO_2 anaesthesia. All females were collected within 6 h of hatching. Females were stored in culture vials containing no live yeast at a density of 16 individuals per vial and allowed to mature for 3 days. On the night before females were used in experiments, their virginity was assured by checking vials for eggs or larvae. Any vials containing eggs or larvae were discarded. Based on a subsample, the average body mass (dry weight) $\pm$ s.e. of small females was 0.194 ± 0.004 mg ($n = 230$) and the average body mass $\pm$ s.e. of large females was 0.402 ± 0.009 mg ($n = 220$). These culture procedures were repeated on each day of the experiments (see §2g).

(d) Fecundity assay of small and large females

To establish fecundity differences between the small and large females, we counted the number of eggs produced by a random subsample of females that were inseminated during the control trials (§2e). Females were left to oviposit for 18 h. Vials were then incubated for 13 days, after which time offspring were counted. This protocol provides sufficient time for virtually all adult flies to emerge (W. R. Rice & A. D. Stewart 2005, unpublished data). However, vials were rechecked on day 16 to ensure that all flies had hatched and that counts were absolute.

(e) Control treatment

To test for the possibility that small and large females differ in their receptivity to male courtship and/or their susceptibility to sexual coercion, we ran trials where 10 males of unmanipulated body size (i.e. males taken from vials reared under standard conditions at a density of 150–200 adults per vial) were introduced into a mating chamber containing either 20 small females or 20 large females. The procedures used to mate flies, culture offspring and score fertilizations were identical to those used to test for male preference (see §2f).

(f) Experimental treatments

(i) Mate preference of non-resource-depleted males

To test whether males showed a preference for larger females, we staged mating trials in which 10 males (3 days old) of unmanipulated body size (i.e. males taken from vials reared under standard conditions at a density of 150–200 adults per vial) were introduced into a mating chamber (10 dram vial with culture medium) that contained 10 small and 10 large females (all 3 days old), (see §2c). The males and females were left together for 30 min. This time was chosen because courtship and mating normally takes 18–20 min, so we gave flies ample time to mate but restricted the opportunity for males to mate twice. Following mating, flies were anaesthetized using CO_2 and removed from the chamber. Females were then housed individually in oviposition vials containing culture medium and approximately 10 mg of yeast to stimulate egg laying and left to oviposit for 48 h before being cleared from the oviposition vials. The vials were then incubated at 25 °C and after 4 days they were examined under a dissecting microscope to determine whether larvae were present. When an oviposition vial contained larvae the resident female was scored as 'mated' and when it was devoid of larvae the resident female was scored as 'unmated'.

(ii) Mate preference of resource-depleted males

To test whether males adjust their mating preference as resources (e.g. sperm supplies and accessory gland products)

available for mating become depleted, we assayed male preference after they had been given the opportunity to mate with multiple females. To acquire 'recently mated males', we placed 10 males and 40 virgin females, all reared under standard culture condition, and therefore of unmanipulated body size (average female body mass (dry weight) $\pm$ s.e. was 0.283 ± 0.0002 mg ($n=300$)), together in a mating chamber for 5 h. Owing to the fourfold excess of females, males were able to mate multiple times during the 5 h period. After this time, males were anaesthetized using CO₂, transferred into fresh vials and left to recover for 1 h. After this treatment, we tested the mate preferences of 'resource-depleted males' following the same protocol used to test the preferences of 'non-resource-depleted' males (see §2f).

(g) Experiment replication and data analysis

We ran a 'primary experiment', and then a second, 'follow-up experiment'. The reason for running the follow-up experiment was twofold. First, in the primary experiment, the effect of the control treatment and the choice treatment was in a similar direction making it difficult to unambiguously separate female mating propensity from male mate choice. Second, in the primary experiment one of the blocks in the 'resource-depleted treatment' showed a pattern (though non-significant) that was in reverse to the overall pattern, so we needed to ensure that this result was not repeatable.

In the primary experiment, we had a mating control to test whether males mated large and small females at similar rates when no choice between female types was available, and two experimental treatments: (i) males that were non-resource-depleted and (ii) males that were resource-depleted (as described previously). Three repeats (blocks) of the experiment were made over three consecutive days. We ran three control and nine experimental trials on day 1, 12 control and 26 experimental trials on day 2 and 8 control and 26 experimental trials on day 3. In the follow-up experiment, we restricted the design to a 'control' treatment and a resource-depleted treatment. Three repeats (blocks) of this experiment were made on three separate days, over a period of 4 days (day 1, 2 and 4). We ran 14 control and 7 experimental trials on day 1, 21 experimental and 20 control trials on day 2 and 15 experimental and 20 control trials on day 4. For both experiments, we used independently derived flies of each sex for each block.

To test whether males mated with small or large females more rapidly under no-choice conditions (controls) and the effects of our experimental treatments on male mate preference (small or large females), we carried out two sample Student's *t*-tests and non-parametric sign-tests. Initially, we fit the full factorial model of remate rate (arcsine transformed) in response to female size (large or small), experimental treatment (resource-depleted, yes or no), block (day 1, 2 or 3, a random effect variable) and testing vial (a random effect variable nested within block and treatment), but this analysis indicated a significant treatment by block interaction, so individual blocks were analysed separately with Student's *t*-tests, and then pooled (using a combined consensus *p*-value test (Rice 1990)) to evaluate overall patterns across blocks. All proportions of females remating were arcsin-square root transformed prior to statistical analysis. Although we predicted males to mate larger, more fecund, females more often than smaller females, all tests of this hypothesis were done with directed tests (Rice & Gaines 1994) rather than one-sided tests to accommodate possible results in the unanticipated direction.

3. RESULTS

(a) Fecundity of small and large females

Females of large body size ($n=92$) produced more than twice as many offspring as females of small body size ($n=85$) (mean $\pm$ s.e. number of offspring: large females = 69.30 ± 1.34 , small females = 25.06 ± 1.41). This difference was highly significant (one-way ANOVA: $F_{1,177}=516.19$, $p < 0.0001$).

(b) Primary experiment

(i) Mating controls

Although males inseminated more large females than small females (large females = $86.66 \pm 3.43\%$, small females = $78.26 \pm 3.28\%$), when there was no choice between the two sizes of females, this difference (8.4%) was not statistically significant (Student's *t*-test, $t=1.7$, $p=0.103$), and was reversed in the follow-up experiment (see below), indicating that female body size was not consistently associated with higher or lower mating rate under the no-choice conditions.

(ii) Experimental treatments

The propensity of males to mate larger versus small females was measured by calculating delta per cent values (percentage of large females mated – percentage of small females mated). Because these estimates varied among different days (blocks) on which the experiment was repeated (ANOVA interaction between blocks and treatment, $F_{2,55}=17.38$, $p=0.03$), we display the data both collectively and within each block individually (figure 1). To provide a robust test of the pattern across blocks, we tallied the number of vials in which the value of delta was positive and negative. Males in the non-resource-depleted treatment mated more frequently with larger females (10 vials had a larger percentage of small females mated and 22 vials had a higher percentage of large females mated, sign-test, $p=0.031$). This same pattern was also observed in the resource-depleted treatment (five vials had more small females mated, 24 vials had more large females mated, sign-test, $p=0.0004$).

We next tested to determine whether the mating bias toward larger females was stronger in resource-depleted males. In block one, where statistical power was lowest due to smaller sample size ($n=9$ vials of 10 males and 20 females), a non-significant reversal of the expected pattern was observed ($t=-1.61$, d.f. = 7, $p=0.871$), but in blocks 2 and 3, where sample sizes were nearly three times larger ($n=26$ vials in each block), there was a significant excess of matings with larger females by resource-depleted males in block 2 ($t=2.10$, $p=0.047$) and a non-significant pattern in this same direction in block 3 ($t=1.66$, d.f. = 24, $p=0.110$). Combining these results with a weighted consensus combined probability test (with weights equalling the number of vials tested in each block), the net result across the three experimental blocks was that resource-depleted males had a significantly elevated bias toward larger females (figure 1a, $p=0.0221$).

(c) Follow-up experiment

(i) Mating controls

Males inseminated a larger percentage of small females compared to large females (*t*-test, $t=2.09$, d.f. = 52, $p=0.0415$; small females = $42.8 \pm 1.9\%$, large females = $34.6 \pm 1.7\%$). This was in the opposite direction from the

Figure 1. The difference in the per cent females mated (mean $\pm$ s.e. of the percentage of large females mated – percentage of small female mated) by male *D. melanogaster*. Data are for (a) a ‘primary experiment’, where males were either ‘non-resource-depleted’ or ‘resource-depleted’ and (b) a ‘follow-up experiment’, where males were only ‘resource-depleted’. For each experiment, data are displayed both collectively and separately within each experimental block (day).

non-significant pattern found for the controls in the primary experiment.

(ii) Experimental treatment

The average delta values were positive for all three experimental blocks (days) (figure 1b). To pool inference across blocks, we scored the number of vials with positive and negative delta values in each block. Resource-depleted males mated large females significantly more often than small females (10 vials had more small females mated and 33 vials had more large females mated, sign-test, $p=0.0006$).

4. DISCUSSION

The results of the primary experiment indicate that resource-depleted males have a stronger preference for large females than non-resource-depleted males. This result was expected based on the knowledge that male *D. melanogaster* invest heavily in mating (Cordts & Partridge 1996). Theoretically, when mating is expensive males can gain a fitness advantage by biasing investment in successive matings towards females of higher reproductive value (e.g. higher fecundity) (Bonduriansky 2001). However, the control treatment, where no choice was available, showed a weak trend toward males mating large

females sooner than small females, so large females may have been more receptive to mating. This result compromised our ability to conclude from the primary experiment that males prefer to mate with larger females, because male choice is confounded with potential differences in the propensity of the two sizes of females to mate.

To address the issue that large females might be more receptive to mating, we repeated the experiment with only the resource-depleted males to focus exclusively on whether males prefer to mate with large females. In this follow-up experiment, we found in the ‘no-choice’ control that small females were mated more rapidly. This pattern was the reverse of that observed in the primary experiment, but it is consistent with a past study examining the association between female body size and mating rate in *D. melanogaster* (Lefranc & Bundgaard 2001). Receptivity of females, like many behavioural traits, is likely to be influenced by many environmental factors besides our body size treatment, so it was not unexpected that this trait might vary among different experiments. The fact that smaller females mated more rapidly in the no-choice controls of the follow-up experiment permitted us to unambiguously assess male choice for larger females in this experiment. Because the control treatment showed that males mated with small females more readily than

large females, but in the 'experimental treatments' males mated large females significantly more than small females, this result provides unambiguous evidence that males prefer larger females, at least when they are expected to be most selective, i.e. when resource-depleted.

In the primary experiment, the observation that larger females mated more rapidly than small females does not affect our conclusion that resource-depleted males had a stronger propensity to mate with large females than non-resource-depleted males. In this case, any difference between the propensity of large and small females to mate was held constant, and, as a consequence, the higher percentage of matings to larger females by resource-depleted males is unambiguously due to increased male preference for large females. This pattern varied in strength and direction in individual replicates, but overall, it was statistically significant when multiple experimental blocks were pooled, so on balance our study provides some of the first empirical evidence to support the theoretical prediction that male stringency in mate choice increases as resources become more limiting (see below).

Taken together, the results of the primary and follow-up experiments suggest that male *D. melanogaster* in our laboratory population can assess the relative size of potential mates and strategically allocate their mating effort in response to variation in mate quality (expected fecundity) and the availability of resources (supplies of sperm and accessory gland products) required for mating (Lefevre & Johnson 1962; Hihara 1981). Given that larger females produced more than twice as many offspring as smaller females, males that preferred larger females gained a direct fitness advantage over males that did not, so discrimination was adaptive. It is important to note, however, that a substantial proportion of 'depleted males' in the experiments still mated with small females. This result suggests that at the level of resource depletion tested (i) the benefit to males of mating with large females may not have consistently outweighed the potential cost of missed mating opportunities (ii) that males assess other phenotypes besides body size in evaluating prospective mates and/or (iii) that males frequently make mistakes. The possibility also needs to be considered that during the depletion process, where males mated for the first time with females that were more similar in body size to small females than large females, a proportion of males may have developed a preference for 'smaller' females. Unravelling these possibilities will require further experimentation. Future work should also focus on examining the mate preferences of males when they are presented with additional female size classes (e.g. medium size females). This data would improve our understanding of the degree of male choosiness.

The results of this study have important implications for mating system theory. Models of sexual selection are in agreement that males stand to increase their fitness by mating with as many females as possible (Bateman 1948; Trivers 1972; Andersson 1994). However, theory also indicates that males should optimize the allocation of limited resources (e.g. time, sperm and ejaculate materials), leading to the prediction that males of species with higher costs of reproduction will bias their mating toward females that will provide the highest reproductive returns (Parker 1983; Gwynne 1991, 1993; Johnson & Burley 1997; Reinhold *et al.* 2002). *Drosophila melanogaster*

now joins a growing list of species where it has been shown that males choose between potential mating partners based on their reproductive value (e.g. Houde 1997; Dosen & Montgomerie 2004). More importantly, our study also suggests that increasing mating costs can influence the stringency of male preference. While there is considerable evidence that female mate choice is sensitive to changes in the costs of being 'choosy' (Jennions & Petrie 1997; Kokko *et al.* 2003), very few studies have attempted to experimentally examine how varying costs affect male mate choice (but see Gwynne & Simmons 1990; Engqvist & Sauer 2001; Wong & Jennions 2003), and none have looked at how male mating decisions respond to changes in male condition, such as the level of sperm depletion. In many species, the costs to males of mating are non-trivial. Therefore, adaptive male mate choice may be more important among animals than is currently appreciated. Lastly, *D. melanogaster* has been used as a model system for many aspects of evolution besides mate choice, including, for example, sexual conflict, gender roles and the evolution of reproductive isolation. Our results indicate that in such studies mating preferences by males cannot be assumed to be of minor importance, and that they are likely to have significant, though previously unappreciated, implications.

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