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Do female *Drosophila melanogaster* adaptively bias offspring sex ratios in relation to the age of their mate?

Tristan A. F. Long* **and Alison Pischedda**

Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Modification of offspring sex ratios in response to parental quality is predicted when the long-term fitness returns of sons and daughters differ. One factor that may influence a mother's sex allocation decision is the quality (or attractiveness) of her mate. We investigated whether the sex ratios of offspring produced by female *Drosophila melanogaster* are biased with respect to the age of the males to which they are mated, and whether there is an adaptive basis for this phenomenon. We found that females mated to old males (13 d post-eclosion) initially produced a greater proportion of daughters than did females mated to young males (1 d post-eclosion). This pattern does not appear to be due to a systematic difference in the numbers or mortality of the X- and Y-bearing sperm originating from old and young fathers, as the overall sex ratios of all offspring produced from a single copulation did not differ between broods fathered by the two types of males. The sons of older males fared worse in competitive mating assays than did the sons of younger males, while daughters of old and young males were of comparable fitness. These results suggest that there is an adaptive basis for the observed sex ratio modification.

Keywords: intraejaculate bias; sperm use; cryptic female choice; sex ratio manipulation; sire age; offspring fitness

1. INTRODUCTION

Sex allocation theory predicts that parents should manipulate the sex ratio of their offspring based on the projected relative fitness of sons and daughters (Trivers & Willard 1973; Charnov 1982). Since the reproductive success (RS) of males is more variable than that of females (Bateman 1948), producing sons instead of daughters is an endeavour that is riskier, but may have greater fitness returns if sons with high RS can be generated. Thus, evolutionary models of sex allocation conclude that producing a disproportionate number of sons will only be adaptive if parents are of sufficient quality to ensure that sons have a high RS, otherwise it may be more beneficial to produce daughters (Trivers & Willard 1973). Although Trivers & Willard initially envisioned this parental quality with respect to maternal condition (and her ability to invest in her offspring), their hypothesis has been expanded to include the possibility that, all else being equal, mothers should adaptively manipulate the sex ratio of their offspring based on the assessment of their mate's qualities (Cockburn *et al.* 2002). If there is heritable variation associated with a trait that affects RS, then females can enhance the fitness of their offspring by producing sons when mated to high quality males and producing daughters when mated to low quality males (Burley 1986; Cockburn *et al.* 2002). Several studies have documented sex ratio patterns consistent with these predictions (e.g. Burley 1986; Ellegren *et al.* 1996; Sheldon *et al.* 1999; Rathburn & Montgomerie 2005), but these results are not universal (e.g. Saino *et al.* 1999; Grindstaff *et al.* 2001).

One interesting case of apparent sex ratio modification in response to paternal condition was reported by Mange (1970) in *Drosophila melanogaster*. In his experiment, virgin females were mated to males of various ages (range 1–15 d post-eclosion), and the sex of all offspring produced from these matings was recorded. This experiment revealed that when females were mated to young males, they initially produced offspring that contained a disproportionate number of sons, while females mated to older males initially produced more daughters than sons. These patterns did not appear to result from differences in the numbers or mortality of X- or Y-bearing sperm that were stored by the female, as the cumulative sex ratios of all the offspring produced from the single mating were equal. The author suggested that females might bias their sperm use, based on male age through the differential storage of sperm in their two types of sperm storage organs (i.e. the seminal receptacle and the spermathecae; Mange 1970).

This research has been cited as potential evidence for intraejaculate cryptic choice by females over which type of sperm fertilizes their gametes (Eberhard 1996, p. 172). Twice-mated *D. melanogaster* females have genetic variability in their likelihood of using the sperm from their first or second mate (Clark & Begun 1998), which might indicate an active role for females in the post-copulatory processes that determine the outcome of competitive fertilization (for alternative interpretations; see Pitnick & Brown 2000). Mange's (1970) observations suggest that sperm selection may also be occurring at a more subtle level. To the best of our knowledge, Mange's (1970) experiment has not been replicated since its publication, and the fitness benefits of biased sex ratios in response to mate age have not been investigated (Simmons 2001).

* Author for correspondence (longt@biology.queensu.ca).

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According to cryptic female choice theory, sperm use biases result from the post-copulatory action of female behaviours, physiologies or morphologies (Eberhard 1996; Pitnick & Brown 2000). In the context of sex ratio modification, this means that females should disproportionately produce offspring of the sex that will have the greatest potential RS (Trivers & Willard 1973; Cockburn *et al.* 2002). If the pattern observed by Mange (1970) is adaptive, we predict that the fitness of sons sired by young males will be greater than the fitness of sons sired by older males (and the corollary true for their daughters).

In this study, we set out to (i) determine whether we could replicate Mange's (1970) test for a temporal bias in the sex ratio of offspring produced by females mated to old and young males and (ii) test whether there were any relative fitness differences between the sons and daughters that are associated with sire age.

2. MATERIAL AND METHODS

(a) *Study populations*

All *D. melanogaster* individuals used in this study came from either a large, outbred, wild-type population (LH_M) or a replicate population (LH_M-*b*) into which a recessive, autosomal marker (*bw*) conveying a brown-eyed phenotype had been introgressed by repeated backcrossing (for details on populations and their histories see Chippindale *et al.* 2001). These populations were maintained in vial culture on a discrete two week generation schedule on Davis cornmeal/molasses media on a 12 h L : 12 h D diurnal cycle at 25 °C in humidity controlled incubators. Both populations were outbred and cultured at large population size (N=1800–2000 adults/generation) and at moderate density. In all experiments we attempted to minimize the effect of larval culture conditions on adult fitness (Pitnick & García-González 2002) by using adult flies that were obtained from vials stocked at a standard (moderate) density of approximately 180 eggs per vial. Adult flies were handled using light CO_2 anaesthesia.

(b) *Experiment 1: assay of sex ratio adjustment in relation to age of mate*

Using the LH_M stock, we obtained males of different ages by sequentially collecting eggs at standard densities for 13 consecutive days. Adult males were collected as they eclosed from their pupae and kept under normal laboratory conditions in single-sex vials of 10 individuals on media that was refreshed every 3 d. Mortality was very low (<1%) in all vials. Using the technique described above, we were able to obtain males that were 1, 3, 5, 7, 9, 11 and 13 d old post-eclosion. These males were mated to 1 d old virgin females that had been placed in groups of 10 in vials containing media and 10 mg of standardized yeast. The sexes were allowed to interact for a period of 2 h, in order to ensure all females had mated once (Long & Pischedda, personal observation). Males were then removed from the vials and discarded, and the all-female vials were returned to the incubator for 24 h. After this time, females mated to 1 and 13 d old males were transferred to newly yeasted vials of media, while those females mated to 3, 5, 7, 9 and 11 d old males were discarded. The vials containing the initial clutch of eggs laid by females mated to males of various ages were placed into incubators and all adult offspring that emerged were counted and scored for sex. We continued to transfer, on a daily basis, the females that had been mated to 1 and 13 d old males to new vials of yeasted media until they stopped producing viable eggs (approximately three weeks). No females died during this time. The vials containing viable eggs were also incubated and all adults that emerged were counted and scored for sex. Thus, we were able to calculate offspring sex ratios for the 'initial' 24 h following copulation (for females mated to males of all ages) and the 'overall' sex ratios for all offspring produced following one copulation (from females mated to 1 and 13 d old males).

(c) *Experiment 2a: assay of individual sex ratio adjustment in relation to age of mate*

As in experiment 1, we collected adult males of different ages (1 and 13 d old post-eclosion) and virgin females (1 d old post-eclosion). This time, however, we collected male and female flies from both the LH_M and LH_M-*b* populations, creating four treatments that differed in both population of origin and sire age (hereafter referred to as old-LH_M, young-LH_M, old-LH_M-*b* and young-LH_M-*b*). For each of these four treatments, we collected 400 adult males and 400 females that were then mass mated. After 2 h, we randomly collected 100 females from each of the four treatments and placed them into individual test tubes that contained media. These females were allowed to oviposit for 18 h after which they were discarded. All offspring that emerged from these test tubes were counted and sexed in order to test whether sex-ratio manipulation occurred at the individual female level.

The remaining mass-mated 300 female flies from each treatment were allowed to oviposit on 35 mm food plates for 18 h, at which time the eggs were collected and used to create two types of experimental vials. These vials contained either exactly 90 old-LH_M and 90 young-LH_M-*b* eggs or exactly 90 young-LH_M and 90 old-LH_M-*b* eggs. Thus each vial contained two types of offspring that differed in both population of origin and sire age. Twenty-nine replicates of each of the two combinations were created and incubated for 11 d (corresponding to the normal culture schedule of these populations), at which time we counted and sexed all the adult flies that had eclosed. Using these flies, we tested for differences in the relative fitness of both sons (using 15 vials from each of the two combinations) and daughters (using 14 vials from each of the two combinations) following the methodologies described below.

(d) *Experiment 2b: assay of relative fitness of sons in relation to sire age*

On day 11 (from egg), all adult flies from our 15×2 experimental vials were transferred to half-pint bottles containing media and 40 mg of yeast to avoid crowding effects before being returned to the incubator for an additional 2 d. At this point, we randomly collected 20 LH_M-*b* females from each bottle (total 300 females per treatment), transferred them individually into test tubes containing media and allowed them to oviposit for 18 h, at which time females were discarded. These eggs were incubated for an additional 14 d and emerging adults were counted and scored for eye colour. The LH_M-*b* females used were homozygous for the recessive *bw* marker, making it possible to determine paternity, as brown-eyed progeny result from fertilization by LH_M-*b* males, and red-eyed progeny result from fertilization by LH_M males.

We determined the relative RS of the two types of males by examining the phenotypes of the offspring from each individual female. Based on the composition of each brood,

it was categorized as being predominantly sired by either LH_M or LH_M-*b* males. If, for instance, a female produced 30 brown-eyed offspring and 10 red-eyed offspring, we concluded that LH_M-*b* males had a greater success than LH_M males while in reproductive competition. The cumulative proportion of broods that were predominantly sired by wild-type males was calculated for each of the replicate vials (15 vials with old-LH_M and young-LH_M-*b* progeny, 15 vials with young-LH_M and old-LH_M-*b* progeny). Thus, by comparing the relative frequency of RS across the four treatments, we were able to simultaneously test for the effects of both sire age and phenotypic marker on the RS of males.

(e) *Experiment 2c: assay of relative fitness of daughters in relation to sire age*

On day 11, the remaining 14×2 vials containing flies sired by males of different phenotypes and ages were used to assay relative female fitness. This was done by transferring eight adult flies of each sex and phenotype (replicated 3 times) from each vial to new conditioning vials containing media and 10 mg of yeast. Flies were incubated for an additional 2 d at which time females were removed and sorted according to eye colour to produce two vials of four LH_M-*b* females and two vials of four LH_M females from each of the conditioning vials. These all-female vials were returned to the incubator for 18 h, and the number of eggs laid during this period was recorded. Egg counts were averaged across vials to provide an estimate of fecundity for LH_M and LH_M-*b* females at the level of the experimental vial. These fecundities were analysed using ANOVA and *t*-tests.

(f) *Experiment 2d: assay of viability and larval survival in relation to sire age*

Twenty-four hours after having been stocked with 90 eggs from two populations, we scored the number of unhatched eggs from each of the treatment vials that were ultimately used to assay the fitness of sons and daughters. These counts were used to calculate egg-to-larvae survivorship for the four treatments. In both experiment 2*b* and *c*, the phenotype and number of all progeny that emerged as adults from the 58 experimental vials was recorded in order to calculate larvae-to-adult survivorship. Egg to adult survivorship was also determined.

(g) *Statistical analysis of sex ratios*

Throughout this study we refer to sex ratio as the proportion of sons produced, rather than the ratio of sons to daughters. To examine sex ratios in relation to sire age we constructed generalized linear models (GLMs) using the statistical package GLMStat X (v 5.7.7 2004; available at http://www.glmstat.com). In each GLM, we used a logit link function and binomial error distribution (Wilson & Hardy 2002) where number of sons is the dependent variable and total number of offspring is the binomial denominator. Significance tests for our models were based on the change in deviance between our data and the null model.

3. RESULTS

(a) *Experiment 1: sex ratio adjustment in relation to age of mate*

Mean sex ratios of offspring produced by groups of females mated to males of various ages over the first 24 h are presented in figure 1. Most treatments resulted in sex ratios that were not statistically different from an equal sex ratio, with the exception of those females mated to 13 d old males, whose offspring sex ratio was female-biased (1377 males/2905 offspring; binomial test, $z=-2.80$, $p=0.006$). There was also a trend towards female-biased sex ratios with increasing sire age across the seven treatments ($r=-0.23$).

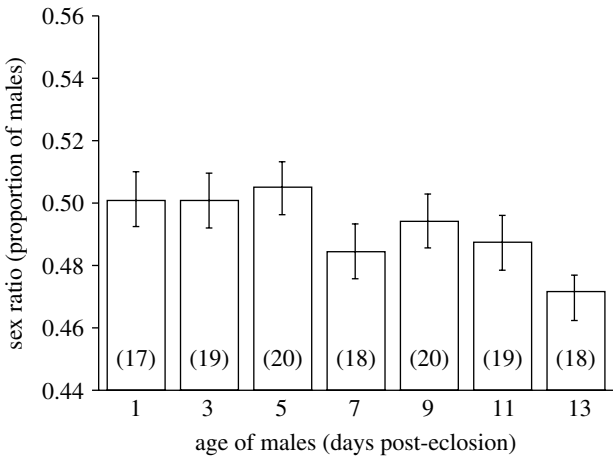

Figure 1. Mean sex ratios (±s.e.) of offspring produced in the first 24 h following copulation by groups of female *D. melanogaster* that were mated to males of various ages. For each column, the number of vials is shown in brackets.

There was a significant difference in the mean (±s.e.) sex ratios of offspring produced by females, in the initial 24 h following copulation, that were mated to 1 d old (0.50 ± 0.007) and 13 d old (0.47 ± 0.008) males ($\chi^2_{1,3}=4.6$, $p=0.03$). There was no difference in the cumulative mean (±s.e.) sex ratios of the broods produced after the first 24 h by females mated to 1 d old (0.49 ± 0.003) and 13 d old (0.49 ± 0.003) males ($\chi^2_{1,33}=0.2$, $p=0.66$). In addition, the overall sex ratios of all offspring produced by females that were mated to 1 and 13 d old males did not differ from each other ($\chi^2_{1,33}<0.1$, $p=0.86$). There was a slight female bias in the overall sex ratios of both groups (mean±s.e.; overall offspring sex ratio from females mated to 1 d old males: 0.49 ± 0.003, one sample *t*-test, $t=-3.50$, d.f.$=16$, $p=0.001$; 13 d old males: 0.49 ± 0.003, $t=-3.29$, d.f.$=17$, $p=0.002$). There was no difference in either the mean (±s.e.) number of offspring produced by groups of females mated to 1 d old and 13 d old males in the first 24 h (young$=173.5\pm12.7$, old$=161.4\pm12.4$; *t*-test, $t=0.68$, d.f.$=33$, $p=0.49$), nor in the total number of offspring produced (young$=1804.7\pm70.0$, old$=1871.7\pm68.0$; *t*-test, $t=-0.69$, d.f.$=33$, $p=0.50$).

(b) *Experiment 2a: assay of individual sex ratio adjustment in relation to age of mate*

Mean sex ratios of offspring produced by individual females mated to 1 d old and 13 d old males in the 18 h following mating are presented in figure 2; these are replicated in both the LH_M-*b* and LH_M populations. Age of mate, but not population (nor their interaction) was a significant predictor of sex ratio variation (table 1). Females mated to young males had male-biased offspring sex ratios, while females mated to old males had female-biased sex ratios. There was no difference in the mean (±s.e.) number of offspring produced by individual females mated to 1 d old

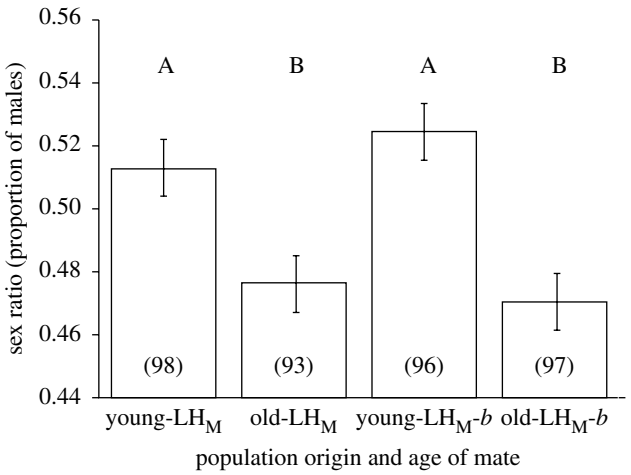

Figure 2. Mean sex ratios (±s.e.) of offspring produced in the first 18 h following copulation by individual female *D. melanogaster* that were mated to either young (1 d post-eclosion) or old (13 d post-eclosion) males in two replicate laboratory fly populations (LH$_M$ and LH$_M$-*b*). Columns that share a letter are not significantly different from each other according to a Tukey's HSD test. For each column, the number of test tubes is shown in brackets. See table 1 for effects of mate age and population origin on offspring sex ratios.

Table 1. Results of a GLM analysis testing the effects of mate age and population origin on offspring sex ratios produced by female *Drosophila melanogaster* (see figure 2 for mean sex ratios).
(whole model: $\chi^2=15.0$, p=0.002, d.f.=3,380. The *z*-ratio represents the change in deviance for each variable. The category associated with a positive estimate is indicated in brackets.)

predictor variable	estimate±s.e.	*z*-ratio	*p*
population origin (LH$_M$-*b*)	−0.08±0.06	−0.26	0.80
mate age (young)	0.11±0.05	2.29	0.02
population origin×mate age	−0.06±0.08	0.83	0.41

and 13 d old males (young=29.1±0.6, old=27.9±0.6; *t*-test, $t=-1.42$, d.f.=382, $p=0.16$).

(c) *Experiment 2b: assay of male fitness in relation to sire age*

The relative RS of males in competition for fertilizations varied depending on both their population of origin and the age of their sire (figure 3). In both age/population combinations, males from the LH$_M$ population secured a greater genetic representation in the progeny of their mates than did those males from the LH$_M$-*b* population. There was, however, a difference in the mean (±s.e.) RS of LH$_M$ males (and by corollary, the RS of LH$_M$-*b* males) that were sired by young males (0.67±0.04) and those that were sired by old males (0.56±0.03; two-tailed *t*-test, $t=2.33$, d.f.=28, $p=0.03$). To ensure that the difference in RS was not an artefact of stochastic differences in the ratio of LH$_M$ and LH$_M$-*b* males competing in each vial, we regressed RS values on the actual LH$_M$ to LH$_M$-*b* male ratios for each of vials, and compared residual values. The difference in RS remained significant ($t=2.11$, d.f.=28, $p=0.04$).

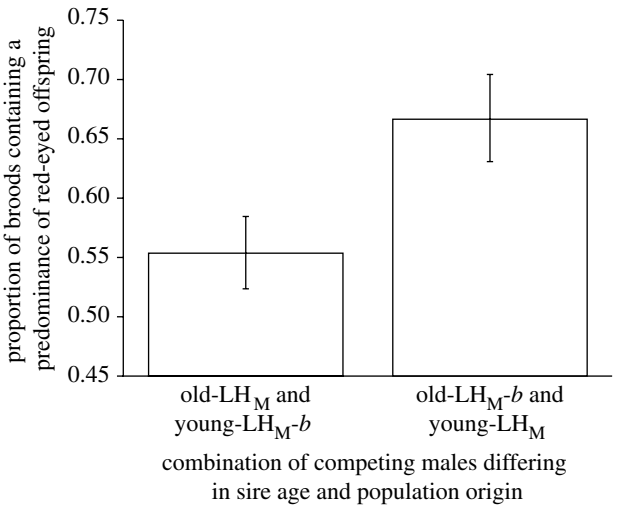

Figure 3. The mean proportion of broods (±s.e.) laid by LH$_M$-*b* females that are predominantly red-eyed from each of two combinations where both red-eyed and brown-eyed males of different sire age compete for fertilizations (i.e. old-LH$_M$ versus young LH$_M$-*b* and young-LH$_M$ versus old-LH$_M$-*b*).

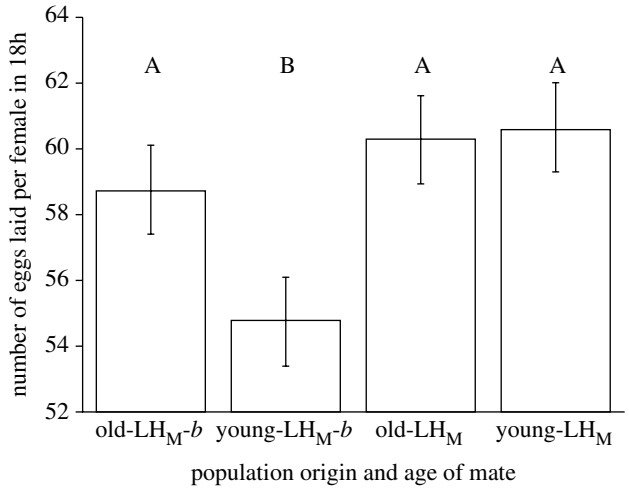

Figure 4. Fecundity of females (mean eggs laid per female over 18 h±s.e.) that were sired by either young (1 d post-eclosion) or old (13 d post-eclosion) fathers in two replicate laboratory fly populations (LH$_M$ and LH$_M$-*b*). Columns that share a letter are not significantly different from each other according to a Tukey's HSD test.

(d) *Experiment 2c: assay of female fitness in relation to sire age*

We performed an analysis of variance on mean female fecundity, with population of origin, age of father and their interaction as the predictor variables. This analysis was significant overall ($F=5.60$, d.f.=3,57, $p=0.002$). Further examination of the effects revealed an interaction between population origin and age of father that was nearly statistically significant at the $p=0.05$ level (population of origin: $F=10.84$, d.f.=1,57, $p=0.002$; age: $F=2.63$, d.f.=1,57, $p=0.11$; population of origin×age of father: $F=3.68$, d.f.=1,57, $p=0.06$). A comparison of mean female fecundities revealed that the daughters of young-LH$_M$-*b* males laid fewer eggs than did the daughters of males from the other three groups (figure 4).

(e) *Experiment 2d: assay of viability and larval survival in relation to sire age*

From the counts of unhatched eggs and total eclosing adults we were able to calculate mean hatchability, larvae to adult survivorship and egg to adult survivorship. We performed analysis of variance tests on these values, with population of origin, age of father and their interaction as the predictor variables. None of the ANOVAs were significant (whole models: hatchability: $F=2.09$, d.f.$=3,117$, $p=0.11$; larvae to adult survivorship: $F=1.25$, d.f.$=3,117$, $p=0.29$; egg to adult survivorship: $F=2.17$, d.f.$=3,117$, $p=0.10$). Overall, the mean ($\pm$s.e.) egg hatchability was 0.97 (±0.02), the larvae to adult survivorship was 0.82 (±0.09) and egg to adult survivorship was 0.79 (±0.08).

4. DISCUSSION

The present study was designed to experimentally address two questions: Does age of mate correlate with sex ratios of offspring produced by female *Drosophila melanogaster*? Are there fitness benefits associated with biased sex ratios? Our results show male age effects on offspring sex ratios and on the relative RS of sons and daughters.

(a) *Male age effects on offspring sex ratios*

This study showed that the offspring sex ratios in the first brood produced by females were biased in relation to the age of the male to which they were mated. In both experiments 1 and 2*a*, females mated to younger males produced initial broods that were either male-biased, or had equal proportions of sons and daughters. Females mated to older males, however, produced initial broods that were female-biased. In experiment 2, these differences were detected in two replicate laboratory populations of *D. melanogaster*. Maternal condition (or 'quality'), which may itself influence offspring sex ratios (see Trivers & Willard 1973), should not have confounded our results, as all females were of the same age and collected under identical culture conditions.

The bias in sex ratio in the initial brood of offspring that are produced immediately following mating does not appear to be due to systematic differences in the mortality or numbers of X- and Y-bearing sperm that are stored following copulations with males of different ages, as there was no difference in the overall sex ratios for all offspring produced by females mated to old and young males. These results are consistent with the original findings of Mange (1970) for short- and long-term patterns in offspring sex ratios. Since there were no differences in hatchability or survival related to sire age or population of origin, and all rates were quite high, it is unlikely that sex and sire age-specific mortality of juveniles is responsible for the observed offspring sex ratios. Our results are in accord with those of Price & Hansen (1998), who were only able to detect a decrease in offspring viability when the father was extremely old (32–33 d post-eclosion), but found no difference between offspring sired by 'young' (2 d old) and 'intermediately aged' (13–14 d old) sires.

(b) *Male age effects on offspring reproductive success*

LH_M males were 19.6% more fit when young fathers sired them than they were when older fathers sired them. This is in accord with the predictions of the Trivers–Willard 'attractiveness hypothesis' (Cockburn *et al.* 2002), which predicts that the RS of offspring produced by females mated to 'high quality' males (in this case young males) will be greater than that of similar offspring sired by 'low quality' (i.e. old) males.

In their study of the effects of paternal age on offspring fitness, Price & Hansen (1998) were unable to detect any differences in the RS of males sired by young (2 d old) and intermediately aged (13–14 d old) fathers. Their assay consisted of competing these males against males carrying the *ebony* mutation for copulations with a virgin female; the first to mate was declared the victor. The nature of their assay, however, may have made it very difficult to detect differences in offspring fitness associated with paternal age. The *ebony* mutation is very deleterious (reviewed in Yamamoto *et al.* 1997), inducing reduced courtship levels and disruptions in courtship behaviour in male carriers. This may explain why the sons of young and intermediately aged males (which were both wild-type) were successful in obtaining the copulation in $>93\%$ of trials when competing with *ebony* males (Price & Hansen 1998). Our assay on the effects of sire age on the RS of males improves upon the approach of Price and Hansen in two fashions. First, we used a genetic marker (*bw*) that is more benign than *ebony*, permitting a more matched competition. Additionally, our assay involved creating sons from old and young sires in both the LH_M and LH_M-*b* replicate populations, permitting us to experimentally tease out the effects of marker, sire age and their interaction on male RS.

The effects of sire age on female RS depended on the population of origin. The *bw* marker appears to slightly lower fecundity compared to wild-type females. LH_M-*b* females that were sired by old males were significantly more fecund than LH_M-*b* females that were sired by young males, while in the LH_M population, female fecundity did not vary with sire age. These results are partially in accord with the prediction that mothers will underproduce offspring of the sex that has the lower fitness returns. For a LH_M-*b* female who is mated to an young male, the fitness benefits of underproducing daughters are two-fold: daughters are less fecund, while sons are more competitive. For a LH_M female mated to a young male, there are no detectable benefits of underproducing daughters, and all benefits of male-biased sex ratios come from the indirect benefits of the greater competitive ability of sons. Overall, it would appear that the fitness of offspring sired by young males is only enhanced by sex ratios that are male-biased.

(c) *Evolutionary significance of sex ratio manipulation*

Why might selection have shaped the sperm use processes of *D. melanogaster* to result in offspring sex ratios that are biased in accordance with the age of the father? Given that over the course of a lifetime, spontaneous mutations in the germ-line of males may arise, there is an increased risk that older males will pass more deleterious mutations to their offspring than younger males. In *D. melanogaster*, the estimated mutation rate for the effective genome (excluding non-coding DNA) is 0.14 per sexual generation (Drake *et al.* 1998). Since sons inheriting deleterious mutations suffer a greater reduction in RS than daughters carrying the same mutations (Spieth 1974; Pischedda &

Chippindale in press), it is logical that either parent should try to decrease the number of low-quality sons produced (when mated to old males) and increase the number of high-quality sons (when mated to young males) in order to maximize their fitness returns. In view of the fact that *D. melanogaster* females typically remate before they have exhausted their sperm reserves (Singh *et al.* 2002), and there is a considerable last-male sperm precedence (reviewed in Simmons 2001), biasing offspring sex ratios over the short term would allow a female to produce more offspring of the sex with the greatest chance of obtaining high fitness returns following each mating.

One limitation of this paper is our inability to demonstrate the mechanism by which offspring sex ratios become biased. Mange (1970) suggested that females bias the storage of X- and Y-bearing sperm in their two types of sperm storage organs (the seminal receptacle and the spermathecae) and differentially use those sperm to alter the fertilization dynamics of their eggs. Because sperm stored in the seminal receptacle is often used first by females for the fertilization of ova (Gilbert 1981; Bloch Qazi *et al.* 2003), altering the distribution of X- and Y-bearing sperm among these organs might produce the observed patterns. We foresee several approaches that could be effectively used to support or refute this hypothesis. One could replicate our experiment in a *Drosophila* species where spermathecae are no longer used for storage (see Pitnick *et al.* 1999). Those species without functional spermathecae may not exhibit the patterns of sex ratio bias described herein. Similarly, one could predict greater sex-ratio biases in *D. melanogaster* mutants producing additional spermathecae (e.g. Bangham *et al.* 2003) and no sex ratio biases in mutants lacking developed spermathecae (e.g. Anderson 1945). Alternatively, the observed sex ratio patterns might result from differences in the order of transfer of X- and Y-bearing sperm, which could be mediated by either sex.

There is growing evidence in many species that females have the capacity to manipulate their stored sperm in order to skew their offspring sex ratios towards that which provides the greatest fitness returns, based on the qualities of their mates. In the side blotched lizard, *Uta stansburiana*, males had a higher rate of survival to maturity when they were sired by large males than by small males, while the survival of daughters was higher when they were sired by small males. Calsbeek & Sinervo (2004) observed that female lizards differentially used their mate's sperm, depending on his size, in order to produce offspring of the sex with the greatest viability. In the white-winged fairy wren, *Malarus leucopterus*, females produced more males when they were paired with males in good body condition (Rathburn & Montgomerie 2005). As in these cases, we have found evidence that offspring sex ratios are biased in correlation to mate quality as well as a potential adaptive basis for this phenomenon. The diagnosis of female cryptic choice, however, is a difficult and controversial task (see Eberhard 1996; Birkhead 1998; Pitnick & Brown 2000), but studies where mate qualities can be experimentally manipulated, biases in sperm use patterns can be confirmed, and fitness consequences of these biases can be assayed, should permit the eventual disentanglement of male and female contributions to sperm use patterns, and in due course, help determine the evolutionary importance of this theory.

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