Sexual Selection and the Random Union of Gametes: Testing for a Correlation in Fitness between Mates in *Drosophila melanogaster*

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abstract: Both males and females vary in fitness. While high-fitness males typically have greater siring success, it is not clear whether these males sire an equal fraction of offspring from all females or a disproportionately large fraction with high-fitness females. The latter nonrandom reproductive pattern can arise as the result of sexual selection and creates a positive correlation in fitness between mates. Such a correlation, if it reflects a positive genetic correlation between mates with respect to fitness, increases the efficiency of selection, reducing mutation load and speeding adaptation. While there is evidence from many taxa that assortative mating for fitness may occur, these studies typically focus on observed matings rather than realized reproductive output. Here, we examine assortative mating for fitness in *Drosophila melanogaster*, first in the context of virgin matings and then using a measure of realized reproduction that incorporates remating and postcopulatory processes. We find evidence for positive assortative mating among virgins but no evidence of assortative mating using the more complete measure of reproduction.

Keywords: assortative mating, sexual selection, condition, fitness, *Drosophila melanogaster*.

Introduction

For males, much variation in fitness comes from variation in siring success (e.g., mating success and success in sperm competition; Clutton-Brock et al. 1988). It is well known that some males have higher siring success than others, though many of the details differ among taxa. Perhaps the most general result is that males in good condition tend to have higher siring success than males in poor condition (reviewed in Andersson 1994; Whitlock and Agrawal 2009). We use the term "condition" in the context of describing differences in fitness that arise from variation in resource acquisition rather than allocation (Rowe and Houle 1996; Tomkins et al. 2004). While much effort has been spent on establishing that males of high condition have increased siring success, considerably less is known about the condition of the females with whom these high-condition males reproduce. Do high-condition males sire a large but equal fraction of offspring from all females, or do these males sire a disproportionately large fraction of offspring from high-fitness females?

The typical theoretical assumption is that male and female gametes combine at random even when sexual selection occurs (e.g., Manning 1984; Whitlock 2000; Agrawal 2001; Siller 2001). Conceptually, the idea is that sexual selection elevates the frequency of gametes from high-condition males in the gamete pool but male and female gametes combine at random thereafter. This assumption greatly simplifies theoretical analyses but is not necessarily correct.

Obviously, the conceptual gamete pool of theoretical population genetics is not a biological reality. Rather than combining at random, there are several biologically plausible reasons why gametes from high-fitness males may tend to combine disproportionately with gametes from high-fitness females, resulting in a positive correlation in fitness between mates. (Note that throughout the text we use the term "mates" to refer to realized mating partners as opposed to those that copulate but fail to produce offspring.) Such a correlation may arise naturally through the two common modes of sexual selection, male-male competition and female choice. A fitness correlation can arise from male-male competition if the intensity of competition increases with female quality, such that poor-quality males are even less likely to win contests for good-quality females than for poor-quality females (e.g., Rowe and Arnqvist 1996). Alternatively, a fitness correlation between mates can occur through female choice. Even if all females prefer high-condition males, a correlation will result if the best females exhibit the strongest preference for the best males (e.g., Bakker et al. 1999; reviewed in Cotton et al. 2006; but see Grether et al. 2005). Moreover, theory predicts that mechanisms leading to a fitness correlation

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should evolve whenever male-male competition or female choice is costly (Fawcett and Johnstone 2003).

There are several consequences of positive correlations in fitness between mates. First, such correlations change how fitness should be calculated. In the absence of a fitness correlation between mates, the fitness of a male may be approximated by the number of offspring sired. If, relative to low-fitness males, high-fitness males reproduce disproportionately with high-fitness females, then the offspring of high-fitness males may be not only more numerous but also of better quality, either because of maternal effects (good mothers make good eggs; Mousseau and Fox 1998) or because high-fitness females pass on good genes to their offspring (Clutton-Brock and Pemberton 2004; Leips et al. 2006; Missoweit et al. 2008). Similarly, a correlation exaggerates the difference in fitness between high- and low-fitness females. If better females reproduce with better males, not only will these females have more offspring but also these offspring will likely have an advantage over offspring from low-fitness females, since the former are more likely to have received good genes from their fathers.

When there is a genetic basis to fitness, a positive correlation in fitness between mates expands the genetic variance in fitness (Fisher 1918). This is because beneficial alleles tend to be clustered in some offspring (the progeny of high-condition parents) and deleterious alleles tend to be clustered in other offspring (the progeny of low-condition parents). This increase in variance allows selection to be more efficient, increasing the rate at which beneficial alleles spread and deleterious alleles are removed. In a theory article, Rice (1998) found that even a weak genetic correlation in fitness between mates can substantially reduce mutation load. This reduced load may provide an advantage to sexual populations over asexual populations (Rice 1998; Jaffe 1999, 2000). In addition, because assortative mating changes the distribution of alleles among individuals, it can also affect the evolution of recombination (Blachford and Agrawal 2006).

There is some evidence for positive correlations in fitness between mates. The most abundant type of data is positive correlations in body size between mates, which have been observed in a number of taxa (see "Discussion"). Such a pattern should result in a positive correlation in fitness between mates, provided a few conditions are met. First, body size must be correlated to fitness, which seems likely for many taxa (Andersson 1994; Kingsolver and Pfennig 2004; but see Irschick et al. 2007). Second, the observed pattern of mating must translate into a correlation in realized reproduction. This requires that there is no bias in which matings are observed, that matings result in sperm transfer, and that postcopulatory mechanisms (e.g., sperm competition) do not swamp out precopulatory patterns. While these conditions may hold true, we are not aware of any examples in which they have been tested.

Here we present data from two experiments in *Drosophila melanogaster* testing for positive correlations in fitness between mates. In both studies, we experimentally created high- and low-fitness flies and then measured whether males and females of these two fitness classes preferentially reproduce with one another. In the first experiment, virgin females from either the high- or low-fitness class were simultaneously exposed to one male from each fitness class in mating trials. This experiment tests for evidence of assortative reproduction based only on a single phase of sexual selection, that is, virgin mating patterns and precopulatory male-male competition. In the second experiment, small mating groups of males and females from both fitness classes interacted over several days. The outcome of this experiment depends on more facets of sexual selection than the first experiment, including virgin matings, rematings, and sperm competition, providing a much more comprehensive view of reproductive patterns.

For both experiments, larval diet manipulation was used to generate high- and low-fitness flies. Because flies reared on a low-quality diet have reduced opportunities to acquire resources, we refer to them as being in low condition (Rowe and Houle 1996; Tomkins et al. 2004). Diet manipulations are commonly used to simulate genetic variation in condition in studies assessing whether sexually selected traits can serve as indicators of genetic quality (reviewed in Cotton et al. 2004). However, it is important to recognize that phenotypic patterns such as those studied here are not necessarily representative of genotypic patterns (e.g., Willis et al. 1991). While caution should be used in extrapolating from any study in which an environmental manipulation is used as a proxy for genetic effects, such manipulations are a useful way to approximate genetic variation in resource acquisition that is likely to be a major source of variation in fitness (van Noordwijk and de Jong 1986; Houle 1991; de Jong and van Noordwijk 1992; Rowe and Houle 1996).

Material and Methods

Experiment 1

The experiment was carried out using a mass-bred stock that originated from a collection made in Dahomey, West Africa, in 1970, which has since been maintained in cage culture at 25°C. To experimentally generate high- and low-condition flies, larvae were reared at low density (~50–80 larvae per 10-dram vial) on high- or low-quality food. The low-quality food medium contained 50% less sugar and yeast than the standard yeast-sugar-agar high-quality me-

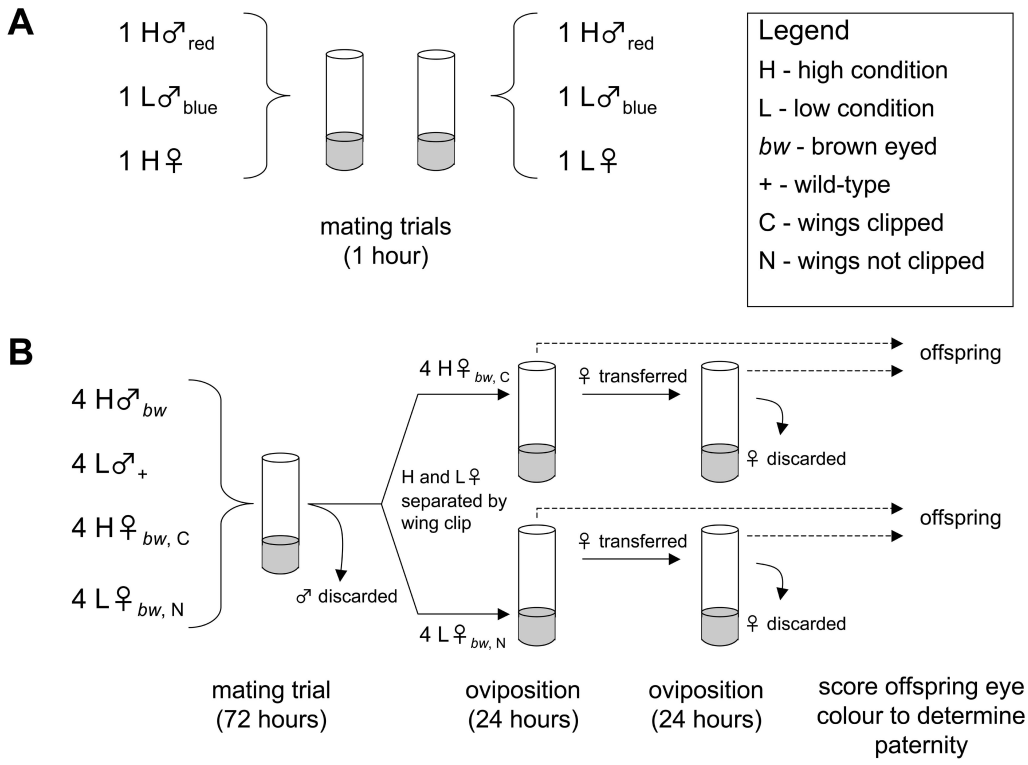

Figure 1: Experimental procedures. *A*, Experiment 1. Each replicate mating trial consisted of one high-condition (H) male, one low-condition (L) male, and one female (either H or L). The first male to mate within 1 h was identified by color (red or blue food coloring). In these examples, H males are marked with red and L males with blue. In approximately half of the replicates, the reverse marking scheme was applied. *B*, Experiment 2. Each replicate mating trial consisted of four H males, four L males, four H females, and four L females for a total of 16 flies. After 72 h, males were discarded, and H and L females were separated by wing clip marking and allowed to oviposit in separate vials for 24 h, flipped into a second vial to oviposit for a further 24 h, and then discarded. The offspring of H and L females were then scored separately, with paternity (H or L sire) determined by eye color (*bw* or wild type). In this example, H males carry the *bw* marker and L males do not, and the wings of H females are clipped, whereas those of L females are not. In approximately half of the replicates, the marking scheme within each sex was reversed, and these marking treatments were applied factorially.

dium. Preliminary studies with this food treatment indicated that females reared on low-quality food were 42% less fecund than females reared on the high-quality food. Flies reared on high- and low-quality food are referred to as being of high (H) and low (L) condition, respectively. Flies reared under the low-quality diet treatment were visibly smaller, and in other experiments employing similar diet manipulations, such flies have been shown to have reduced mass or size (Byrne and Rice 2006; Amitin and Pitnick 2007; McGraw et al. 2007; N. P. Sharp, unpublished data). Larvae were randomly assigned to vials of high- or low-quality food approximately 24 h after hatching.

Adults were collected 8–12 h after emergence using CO_2 anesthesia. Virgins were housed in single-sex vials containing 25 flies per 10-dram vial, which contained standard yeast-sugar-agar media seeded with live yeast. The day of virgin collection is termed day 0. On day 2, females were placed into individual vials using light CO_2 anesthesia (<10 s). To allow H and L males to be distinguished, they were marked by allowing them to ingest either red or blue food coloring; to do this, on day 2, males were transferred to new vials containing live yeast stained with food coloring. The assignment of food coloring to treatment was alternated.

Mating trials were performed on day 3. One H male and one L male were combined into a single vial using light CO_2 anesthesia. Five to 10 min later, each pair of males was moved to a vial containing a single female (either H or L) without anesthesia, thus initiating the mating trial (fig. 1*A*). All mating trials occurred at 25°C under lighted conditions. Vials were spot checked every 5 min for mating. If mating was observed, flies were killed and the color of the mating male recorded. There were a few cases where male color could not be identified, and these trials were excluded from analyses. If no mating was observed after 60–70 min, the trial was terminated.

Experiment 2

In addition to the outbred population described above, a version of this population carrying a visible genetic marker was used in experiment 2. The recessive brown (*bw*) allele was introgressed into this outbred background through 10 generations of serial backcrossing. Large stocks (at least 2,500 adults per generation) of wild-type and *bw*/*bw* flies were maintained in identical culture conditions in vials for several generations before experimentation.

In this experiment, small mating groups were created, each containing both high- and low-condition males and females. We clipped the wings of some females to distinguish between the female types, and the *bw* eye color allele was used to distinguish between the male types. The procedure for experiment 2 is summarized in figure 1*B* and described in detail below.

High- and low-condition flies were created by placing first instar larvae at a density of exactly 50 per vial on either standard food or food containing 50% less yeast and sugar. Both wild-type and *bw*/*bw* flies were reared under these two diet treatments. The experiment was carried out in three blocks. For each block, all larval transfers onto low food were done on a single day (day T); the larval transfers onto high food were done over 2 days (day T and day $T + 1$) because of the slightly faster and more synchronous development of flies on high-quality food.

From the *bw*/*bw* vials, H and L males and females were collected on emergence. From the wild-type vials, only H and L males were collected. Flies were collected as virgins over a 2-day period, using the H vials generated on day T on the first day of virgin collection and H vials from day $T + 1$ on the second day of collection; L virgins were collected on both days from the L vials generated on day T. This was necessary because L flies tend to emerge over a 2-day period, whereas H flies tend to emerge on a single day. H and L flies of a particular sex were always the same age (days posteclosion) within a replicate mating group. Flies were housed on standard media seeded with live yeast at a density of 15–20 individuals per 10-dram vial and maintained under standard lab conditions (25°C, 12L : 12D) for 2–4 days before the mating trials.

Each replicate mating group consisted of four H males, four L males, four H females, and four L females. Our goal was to estimate the reproductive success of each male type with each female type. Accordingly, we used the genetic marker *bw* to track whether offspring were sired by H or L males. In approximately half of the replicate mating groups, the H males were homozygous for the *bw* marker. L males carried the marker in the remaining replicates.

To determine the relative success of each male type with each female type, we physically marked one female type by clipping wings. This allowed us to separate the female types following mating, such that they produced offspring in separate vials. In approximately half of the replicate mating groups, we clipped the wings of H females. The wings of L females were clipped in the remaining replicates. Wing clipping involved removing a small amount of the distal end of one or both wings using microscissors. Clipping was performed on a random subset of collected virgin females 1 or 2 days after collection. Previous studies have shown that wing clipping has no effect on female mating success (Averhoff and Richardson 1974), and we did not find any effect of wing clipping on fitness here (see "Results"). Because the *bw* allele is recessive, all females used in every replicate were homozygous for *bw*.

These male and female marking treatments were performed in a factorial manner, resulting in four different mating group treatments, which are outlined in table 1. For example, in the first type of mating group, the *bw* marker was carried by H males (L males were wild type), and the wings of H females were clipped (L females' wings were not clipped). Although we have labeled these as treatments A, B, C, and D for ease of discussion, male and female marking schemes were treated as separate factors in the analysis. An approximately equal number of A, B, C, and D replicates were performed within each of the three experimental blocks.

Mating trials took place over 3 days on standard yeast-sugar-agar media, seeded with live yeast. Studies of remating in *Drosophila melanogaster* suggest that the majority of females will likely mate multiply within this period of time (e.g., Prout and Bundgaard 1977; Scott 1987; van Vianen and Bijlsma 1993; Stewart et al. 2005). After 3 days in the mating trials, females were removed under light

Table 1: Summary of mating group treatments for experiment 2

	Male	Female
A:		
H	*bw*	*bw* (C)
L	+	*bw* (N)
B:		
H	*bw*	*bw* (N)
L	+	*bw* (C)
C:		
H	+	*bw* (C)
L	*bw*	*bw* (N)
D:		
H	+	*bw* (N)
L	*bw*	*bw* (C)

Note: A–D are the four mating group treatments, each of which contain four high-condition (H) and low-condition (L) males and females for a total of 16 flies. C = females whose wings were clipped before the mating trial; N = nonclipped females; *bw* = *bw*/*bw* males; + = +/+ males. Females were always *bw*/*bw*.

CO_2 anesthesia, and males were discarded. Any deaths that had occurred during the mating trial were noted at this time. One or more females (typically just one) were found dead in approximately 15% of the replicates. One or more males (typically just one) were found dead in approximately 9% of the replicates. From each replicate, clipped and nonclipped females were separated, placed in groups in yeasted vials to lay eggs over 24 h, moved to new vials to lay eggs for a further 24 h, and then discarded, resulting in a total of four oviposition vials per mating group (two for H females and two for L females). To determine paternity, offspring from these vials were scored with respect to eye color: *bw*/+ flies had red eyes and *bw*/*bw* flies had brown eyes. Each vial was scored twice, first on day 12–14 and then again on day 15. These values were summed to obtain a total for that vial. Offspring were discarded after being scored.

To summarize, each mating trial consisted of males and females raised in high- and low-condition food treatments. H and L females were separated before egg laying on the basis of clipped or nonclipped wings, allowing us to estimate the number of offspring produced by both types of females. By scoring the *bw* allele, we could determine the fraction of offspring produced by each female type that were sired by H versus L males. Thus, groups of 16 flies were allowed to interact freely for 3 days, and our data reflect patterns of realized reproduction as estimated by the offspring produced over the subsequent 2 days (fig. 1*B*). Replicate mating groups where either type of female entirely failed to produce offspring were excluded from all further analyses. In total, four out of 441 replicates were excluded for this reason.

Results

Experiment 1

In the first experiment, a total of 819 mating trials were performed (427 with low-condition females and 392 with high-condition females). Mating occurred in 544 of these trials. Mating rates were significantly higher among high-condition females: mating occurred in 277/392 = 71% of trials with H females and in 267/427 = 63% of trials with L females (χ^2 contingency test using Yates correction for continuity: $\chi^2 = 5.70$, df = 1, $P < .05$).

The numbers of matings with males of each type are shown in table 2. We tested whether H or L males were more likely to mate first with high- and low-condition females using χ^2 goodness-of-fit tests, where each male type was expected to mate first 50% of the time (Zar 1984). H males won contests more often than L males when placed with either low-condition females (146/259 = 56%, $\chi^2 = 4.20$, df = 1, $P < .05$) or high-condition females (182/276 = 65%, $\chi^2 = 28.06$, df = 1, $P < 10^{-6}$). Moreover, the proportion of matings with high-condition males was greater for high-condition females than for low-condition females (χ^2 contingency test using Yates correction for continuity: $\chi^2 = 4.78$, df = 1, $P < .05$). These results indicate that high-condition males have an advantage over low-condition males and that this advantage is greatest in trials involving high-condition females. As in other studies (Rundle et al. 1998; Dolgin et al. 2006), the food coloring used to mark males in this experiment (blue vs. red) did not have a significant effect in trials with either high- or low-condition females ($P = .59$ and .14, respectively).

Table 2: Number of mating trials in which low- and high-condition females mated with low- versus high-condition males in experiment 1.

Condition of female	Condition of mated male		Trials with successful high-condition male (%)
	Low	High	
Low	113	146	56.4
High	94	182	65.9

Note: Percentages are within each female condition, because each mating trial consisted of two males and one female. χ^2 tests were performed on each row individually as well as on the entire 2 × 2 table (see "Results").

Experiment 2

In the second experiment, a total of 441 replicate mating groups were examined, from which more than 168,000 offspring were scored (four replicates were excluded from analyses). To determine whether females reared on high-condition food were more fit than females reared on low-condition food, we calculated the difference in the number of offspring produced by H and L females within each replicate, Δ_F. This response variable was analyzed in a linear model with eye color of the H males, wing type of the H females, and their interaction as fixed effects and experimental block as a random effect. The intercept from the linear model was large and positive (intercept estimate ± SE = 103.7 ± 4.4, $P < .0001$), indicating a strong fitness advantage of H females over L females. Although block was significant ($P < .001$), there was always a large advantage of H females over L females in all blocks (least squares means for Δ_F: block 1, 94.5; block 2, 127.8; block 3, 88.9). Neither H male eye color nor H female wing type had a significant effect on Δ_F. Averaging over all treatments and blocks, L females were ~44% less fecund than H females (fig. 2), confirming that our diet treatment was successful in generating differences in female fitness.

The effect of the diet manipulation on male fitness was assessed in a similar manner by calculating the difference

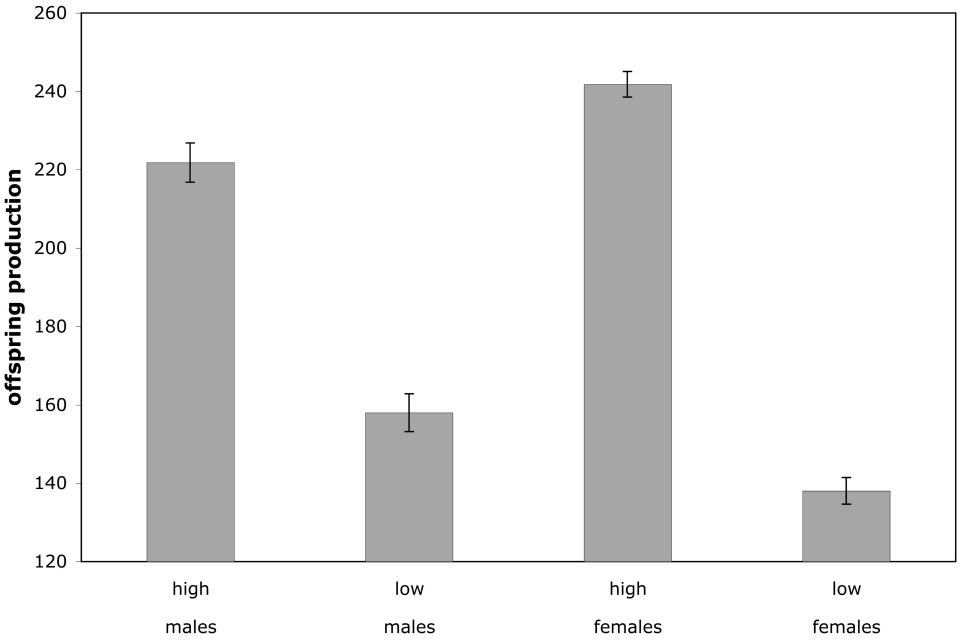

Figure 2: Least squares means ± SE of offspring production by high- and low-condition males and females in experiment 2, averaged over all mating group treatments and blocks. High-condition males had greater siring success than low-condition males. High-condition females had greater fecundity than low-condition females.

in the number of offspring sired by H and L males within each replicate, Δ_M. As with females, the intercept term was strongly positive (intercept estimate ± SE = 63.9 ± 8.5, $P < .0001$), indicating a large advantage of H males over L males. Neither block nor H female wing type significantly affected Δ_M. However, there was a significant effect of H male eye color ($P < .0001$). When H males were *bw/bw*, their advantage over L males was much smaller (LS mean Δ_M ± SE = 29.6 ± 11.5) than when H males were wild type (LS mean Δ_M ± SE = 98.1 ± 12.2). These results suggest that there was sexual selection against the *bw* mutation, as has been previously reported (Stewart et al. 2005). Although selection against this marker introduced unwanted variation into the experiment, H males tended to sire more offspring than L males regardless of eye color. Averaging over all treatments and blocks, L males sired ~28% fewer offspring than H males (fig. 2), confirming that our diet treatment was successful in generating differences in male fitness.

The primary goal of this experiment was to determine whether H males sired a larger fraction of the offspring from H females than from L females. For each replicate we calculated $\Delta_p = p_{HH} - p_{HL}$, where p_{HH} and p_{HL} are the fractions of offspring that were sired by H males produced by H and L females, respectively. A value of $\Delta_p = 0$ means that H males were equally successful with H females and L females. A positive value of Δ_p means that H males had better success with H females than with L females, which would indicate a positive correlation in fitness between mates. The intercept of the linear model is very close to 0 (intercept estimate ± SE = 0.009 ± 0.018, $P = .62$), indicating that the siring success of H males does not differ between H and L females. There was a significant effect of H male eye color ($P < .01$) on Δ_p. As shown in figure 3, average Δ_p values tended to be slightly positive in mating groups where H males were *bw/bw* and slightly negative in mating groups where H males were +/+. This indicates that, contrary to the expectation if there were positive assortative mating for fitness, the trend was for Δ_p to be slightly negative in those treatments where the difference in fitness between H and L males was the greatest.

Discussion

Most theory assumes that mating is random, but that assumption has rarely been tested experimentally. Moreover, there are reasons to believe that positive correlations in fitness between mates may be common (Fawcett and Johnstone 2003). Such a correlation can expand the variance in fitness and increase the efficiency of selection, leading to faster adaptation and reduced mutation load (Fisher 1918; Rice 1998).

One way to look for such a correlation is to ask whether high-fitness males have the same success rate with high-

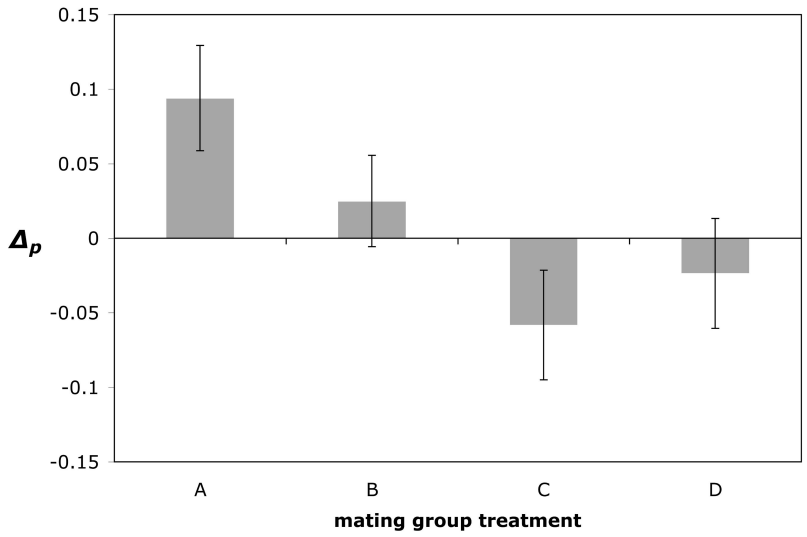

Figure 3: Effect of the different marking treatments on Δ_p estimates. Mean $\Delta_p \pm$ SE for each mating group treatment in experiment 2. $\Delta_p = p_{HH} - p_{HL}$, where p_{HH} and p_{HL} are the fractions of offspring that were sired by high-condition (H) males produced by H and low-condition (L) females, respectively. $\Delta_p = 0$ indicates that H males have equal siring success with H females and L females. $\Delta_p > 0$ indicates that H males have better success with H females than with L females, that is, a positive correlation in fitness between mates.

fitness females as they do with low-fitness females. If these rates are the same (i.e., $p_{HH} = p_{HL}$ or $\Delta_p = 0$), then there is no correlation and gametes can be thought to be combining at random. If high-fitness males are more successful with high-fitness females than they are with low-fitness females (i.e., $p_{HH} > p_{HL}$ or $\Delta_p > 0$), then gametes do not combine at random and there is a positive correlation in fitness between mates. We experimentally created high- and low-fitness classes of males and females and asked whether they preferentially reproduce with one another by measuring Δ_p.

In experiment 1, we found that high-condition males were the first to mate in more trials than low-condition males with both high- and low-condition females (i.e., $p_{HH} = 0.66 > 1/2$ and $p_{HL} = 0.56 > 1/2$). More important in the current context, high-condition males were more successful with high-condition females than with low-condition females (i.e., $p_{HH} > p_{HL}$, $\Delta_p = 0.09$). In other words, low-condition males always suffered a selective disadvantage, but the strength of selection was mediated by female condition. The selection against low-condition males was about twice as strong in the context of high-condition females ($s_H = 0.46$) as in the context of low-condition females ($s_L = 0.23$). (These heuristic selection estimates are based on the classic evolution equation $p_x' = p_x W_x / \overline{W}_x$, where p_x and p_x' are the frequencies of type x (high or low) before and after selection, respectively, and W_x is the fitness of type x. For high- and low-condition males, we use $W_H = 1$ and $W_L = 1 - s$).

Experiment 1 was limited in several ways. It involved mating trials between virgin flies and therefore completely excluded other phases of sexual selection, such as remating and sperm competition. Moreover, the trials lasted ~1 h, and no matings were observed in this period in 30%–40% of trials; it is impossible to know whether the eventual outcomes of those trials would have differed from the observed patterns. Finally, each of the trials involved only three flies: a single female (H or L) and a male of each type. This design limits the scope for some behavior. There is no opportunity for males to exhibit preference in whom they court (Bonduriansky 2001; Byrne and Rice 2006) or to target a different female if they are being outcompeted for their initial target.

Experiment 2 avoided many of the limitations of experiment 1 and was much larger in scale. We were able to confirm that the larval diet manipulation had the desired effect on fitness in both sexes. Relative to high-condition flies, low-condition females were ~44% less fecund, and low-condition males sired ~28% fewer offspring. In contrast to experiment 1, we found no evidence in experiment 2 that high-condition males were more successful with high-condition females than with low-condition females. This negative result does not stem from a lack of power: our point estimate for the mean difference was $\Delta_p = 0.009$, and the standard error was small (SE = 0.018), suggesting that there is at most a very small difference in the success rates of high-condition males with high- and low-condition females. Moreover,

simulations (not shown) incorporating elements of the design of this experiment and relevant aspects of fly biology (e.g., strong last-male sperm precedence) suggest that our power to detect differences even half as large as those observed in experiment 1 (e.g., $\Delta_p \approx 0.05$) was very high ($1 - \beta > 0.9$).

The results of experiment 1 suggest that a positive correlation in fitness between mates is likely. However, experiment 2 found no evidence of a correlation in fitness, and this is the most relevant study because it more completely captures all aspects of reproduction. It remains a challenge to determine what aspects of reproductive success present in experiment 2 but missing from experiment 1 (e.g., remating, sperm competition) are responsible for the apparent difference in the results.

The absence of support for a correlation in fitness between mates in experiment 2 contrasts with other studies in the literature. Assortative mating patterns have been identified in a wide variety of animals, including reptiles (Shine et al. 2001), amphibians (Robertson 1990; Gutierrez and Luddecke 2002), fish (Maekawa et al. 1994; Silva et al. 2008), birds (Olsen et al. 1998; Forero et al. 2001), mammals (Preston et al. 2005), insects (McCauley and Wade 1978; Hieber and Cohen 1983; Johnson 1983; Snead and Alcock 1985; McLain and Bromisia 1987; Brown 1990; Otronen 1993; Arnqvist et al. 1996; Bonduriansky and Brooks 1998; Harari et al. 1999), and other taxa (Greenspan 1980; Erlandsson and Rolan-Alvarez 1998; Monroy et al. 2005; Pal et al. 2006). These studies find evidence for assortative pairing or copulation for body size or mass under field conditions, but this is by no means an exhaustive list of such studies. Territoriality can lead to assortative mating for competitive ability if there is intrasexual competition among females and males for access to the best territories (Creighton 2001). Assortative mating has also been observed in laboratory studies of some *Drosophila* species (*Drosophila malerkotliana*: Hegde and Krishna 1997; *Drosophila ananassae*: Sisodia and Singh 2004). These studies focus on the mating behavior of small groups of individuals but do not examine the outcome of multiple matings and sperm competition.

It is unclear why our result does not match these other studies. Correlations may be ubiquitous in nature but absent under the experimental conditions used here, or observational studies in which no significant correlation was detected (e.g., Dickerson et al. 2004) may be less likely to be published (Palmer 2000). A more interesting possibility is that the correlations reported in the studies above may not actually represent correlations in fitness between mates. These studies focus on body size, and the actual correlation in fitness between mates may be weaker than the correlation in body size. In addition, these studies often assume that the observed matings result in successful insemination and that the observations were not biased. The potential for a discrepancy between observed mating patterns and realized reproduction has been powerfully demonstrated in studies of avian mating systems, where ostensibly monogamous species proved to have high levels of extrapair paternity when actual reproductive output was assessed by molecular markers (Petrie and Kempenaers 1998). Moreover, even if observed mating patterns are not a biased sample, it should be noted that assortative copulation does not necessarily constitute assortative reproduction; postcopulatory processes might enhance or negate a pattern of assortative copulation.

As discussed earlier, a positive correlation in fitness between mates has several major implications, on the condition that it reflects not just a phenotypic correlation but also a genetic correlation between mates with respect to fitness. Such a correlation expands the variance in fitness (Fisher 1918), increasing the efficiency of selection. Thus, this type of reproductive pattern can alter predictions regarding the rate of adaptation, mutation load, and the evolution of sex and recombination (Rice 1998; Blachford and Agrawal 2006). Moreover, a correlation in fitness between mates can affect how we should estimate fitness (e.g., offspring number may misrepresent male fitness if good males, in addition to siring more offspring, also sire offspring of better quality by mating with high-quality females). It is implicitly assumed in many theoretical models and empirical studies that no correlation in fitness exists between mates even though a considerable number of studies report positive assortative mating for body size, indicating that such a correlation could be common. However, in our own study, the observed mating pattern did not match the pattern estimated from a more complete measure of realized reproduction. In interpreting our results, it is important to bear in mind that we have used an environmental manipulation to create high- and low-fitness classes as a surrogate for mutationally unloaded and loaded individuals. While we believe this approach is reasonable, patterns of mating based on "genetic quality" might differ from those measured here. More comprehensive studies of reproductive patterns are needed to address these issues in additional taxa and under natural conditions.

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