

Mating Frequency and Inclusive Fitness in *Drosophila melanogaster*

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ABSTRACT: In many species, increased mating frequency reduces maternal survival and reproduction. In order to understand the evolution of mating frequency, we need to determine the consequences of increased mating frequency for offspring. We conducted an experiment in *Drosophila melanogaster* in which we manipulated the mating frequency of mothers and examined the survival and fecundity of the mothers and their daughters. We found that mothers with the highest mating frequency had accelerated mortality and more rapid reproductive senescence. On average, they had 50% shorter lives and 30% lower lifetime reproductive success (LRS) than did mothers with the lowest mating frequency. However, mothers with the highest mating frequency produced daughters with 28% greater LRS. This finding implies that frequent mating stimulates cross-generational fitness trade-offs such that maternal fitness is reduced while offspring fitness is enhanced. We evaluate these results using a demographic metric of inclusive fitness. We show that the costs and benefits of mating frequency depend on the growth rate of the population. In an inclusive fitness context, there was no evidence that increased mating frequency results in fitness costs for mothers. These results indicate that cross-generational fitness trade-offs have an important role in sexual selection and life-history evolution.

Keywords: multiple mating, inclusive fitness, parental effects, sexual selection, sexual conflict, maternal effects.

In short, the counter-intuitive possibility exists that females with lower than average lifetime reproductive success may actually be fitter. (Kokko et al. 2003)

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Sexual behavior is a great evolutionary paradox. In 1948, William Bateman (1948) showed that the marginal return of a mating event, measured by changes in lifetime reproductive success (LRS), is roughly constant for male fruit flies but decreases with every additional mating for females. This work suggested that the optimal mating rate is higher for males than for females. In the 1970s, work by Trivers (1972) and Parker (1979) indicated that these reproductive conflicts of interest between males and females might, paradoxically, drive the evolution of reduced female fitness. This hypothesis was supported by the finding that increased mating substantially reduces the LRS of mothers in at least 15 different species of insects (Arnqvist and Nilsson 2000). In addition, manipulative studies and selection experiments in *Drosophila melanogaster* show that mating and exposure to compounds in male seminal fluid can reduce the survival and fecundity of females (Cohet and David 1976; Fowler and Partridge 1989; Chapman et al. 1995; Rice 1996; Holland and Rice 1999; Brown et al. 2004).

There are two explanations for why sexual selection can reduce female LRS (Pizzari and Snook 2003). Sexual conflict theory predicts that sexual selection on traits in males might cause reduced LRS in females (Chapman et al. 2003; Arnqvist and Rowe 2005). Alternatively, mutualistic theories, such as sexy son, good genes, and genetic compatibility models, have primarily focused on the possibility that sexual selection might allow for the evolution of reduced maternal LRS if it improves the genetic constitution of offspring (reviewed in Jennions and Petrie 2000; Zeh and Zeh 2003). Still, despite more than 2 decades of empirical investigation that have established that multiple mating behaviors and costs of mating are widespread in the animal kingdom, there is no clear consensus about how mating behaviors that reduce maternal LRS can evolve (Arnqvist and Nilsson 2000; Kokko et al. 2003; Zeh and Zeh 2003).

It is possible that LRS may not be the most appropriate fitness measure for evaluating mating success (McGraw and Caswell 1996; Brommer 2000; Brommer et al. 2002). Though the parental LRS approach combines survival and

fecundity components of fitness into a single useful measure, it assumes that the population has a growth rate of zero. When the population growth rate (often referred to as m , the Malthusian parameter) is nonzero, the age-specific patterns of survival and fecundity can affect how many descendents a cohort will have in subsequent generations (Cole 1954; Charlesworth 1970; Mertz 1971; Caswell 2001). As such, m can be used as a metric of fitness under deterministic, density-independent conditions (Lotka 1939; Charlesworth 1994). When m is positive, early-life reproduction makes the greatest compounded contribution to fitness, and conversely, when m is negative, late-life reproduction contributes the most to fitness. The LRS and age-specific m approaches often yield remarkably different estimates of the relative success of different cohorts or genotypes (Brommer et al. 2002). For example, in fruit flies, increased male exposure decreased fitness by 20% when m was assumed to be 0 (the LRS assumption) but only by 4% when m was assumed to be large and positive (Tatar and Promislow 1997).

However, using LRS and m as estimates of fitness can be misleading when there are parental effects, which occur when traits expressed in parents influence traits expressed in offspring, independent from offspring genetic effects (Mousseau and Fox 1998). If maternal LRS is correlated with offspring LRS, then a cross-generational approach must be used to accurately estimate maternal fitness (Grafen 1988). Hamilton (1964) developed the theory of kin selection to explain the evolution of behaviors that are detrimental to individual fitness. Hamilton's rule states that an altruistic behavior can evolve when the fitness costs (c) associated with the behavior are less than the fitness benefits (b) to relatives times the degree of relatedness (r) between the actor and the benefiting relatives, $c < rb$. Hamilton's rule could apply to the evolution of costly mating behaviors in fruit flies if maternal mating improves offspring fitness.

There are two questions that must be answered in order to evaluate whether cross-generational fitness trade-offs influence the evolution of sexual behavior. First, how does maternal mating frequency affect the survival and fecundity of mothers and their daughters? Second, are the fitness costs of increased mating frequency for females balanced by fitness benefits to daughters in an inclusive fitness context? In this study, we addressed these questions through manipulative experiments using *D. melanogaster*. We manipulated maternal mating frequency and examined the effect of that manipulation on the age-specific survival and fecundity of mothers and their daughters. We then combined these demographic estimates using an inclusive fitness approach that accounts for population growth rate to evaluate the cross-generational effect of mating on female fitness.

Material and Methods

Maternal mating frequency was manipulated, and its influence on the survival and fecundity of mothers and the survival and fecundity of their daughters was examined. We used the Dahomey strain of *Drosophila melanogaster*, which was caught in Dahomey, West Africa, in 1970 and since then has been maintained as a large (>1,000 flies) outbred strain in population cages (Partridge and Andrews 1985). We cultivated flies at 50 eggs/vial for three generations before the start of the experiment to equalize differences in maternal environmental effects and to account for egg density effects on fitness traits (Clare and Luckinbill 1985). For each generation of culture and for all of the vial transfers, we used standard fly media without additional dry yeast.

Maternal Generation

We studied the effects of mating frequency on maternal fitness in three replicate trials that were initiated in consecutive 2-week intervals. For each trial, we collected virgin females (the mothers) from strain culture vials over a 5-h collection interval. In total, we collected 557 mothers (192, 185, and 182 for trials 1, 2, and 3, respectively). Each mother was placed in her own cage. Each cage (roughly half the size of a standard fly media vial) had a rubber gusset for aspirating flies on one end and a flexible plastic sleeve for removing and adding fly media vials on the other end. After 48 h, at maternal age day 2, three virgin males (2–4 d old and chosen from a large pool) were added to each cage. For each trial, cages were distributed in a randomized block design in eight trays, which were treated as blocks in the statistical analyses. Each block contained between 22 and 24 cages. The cages in each block were randomly assigned to one of three mating treatments: high, medium, or low. Most of the blocks had eight replicates per treatment per block; none had fewer than six replicates for a specific treatment. For trial 1, there were 62 high-, 63 medium-, and 67 low-mating replicates. For trial 2, there were 60 high-, 63 medium-, and 64 low-mating replicates. For trial 3, there were 57 high-, 61 medium-, and 64 low-mating replicates.

The basic mating design was similar to that of previous studies (Fowler and Partridge 1989; Brown et al. 2004). The low-mating mothers were exposed to three virgin males for 1 day every 6 days from maternal age 2 through 50. The medium-mating mothers were exposed to three virgin males for 1 day every 3 days from maternal age 2 through 53. The high-mating mothers were exposed to males every day from maternal age 2 through 53. For each 3-day interval, high-mating mothers received fresh males on the first and second day, and the second set of males

was retained for the third day. The males were discarded after each mother exposure. Providing mothers with virgin males helped ensure uniformity among males (Torres-Vila and Jennions 2005), and providing mothers with multiple males allowed females to choose mates.

The mothers were transferred to new vials every other day until death. The vials in which mothers laid eggs were retained for fecundity analyses. From the age at death values, we calculated longevity and age-specific mortality rate (μ ; Elandt-Johnson and Johnson 1980). To estimate age-specific fecundity, we counted the number of hatched pupal cases in every other sequential vial collection (collections 2, 4, 6, etc., which correspond to maternal ages 3–4, 7–8, 11–12, etc.). Because we did not count half of the collections, we estimated the total number of offspring produced by each mother, maternal LRS, by multiplying the number of counted offspring by 2. To determine whether the alternate-day sampling procedure for fecundity provided a good representation of the total fecundity, we counted all of the hatched pupal cases for all of the mothers in trial 3. The correlation between the alternate-day and full-sampling regimes was $r = 0.80$ ($n = 165$). In order to estimate egg-to-adult viability, we counted the number of eggs that each mother laid in every fourth 2-day interval (collections 4, 8, 12, etc., which correspond to maternal ages 7–8, 15–16, 23–24, etc.) and compared that to the number of hatched offspring these vials produced. During the course of the experiment, 1.8% of all of the maternal flies (10/557) escaped or were lost as a result of handling errors. These flies were censored in the survival analyses and eliminated from the lifetime reproductive success analyses but were included in the egg viability and age-specific fecundity analyses for the intervals before they were lost.

Daughter Generation

We collected daughters from maternal trial 3 to study the effects of maternal mating frequency on daughter fitness. Mating and exposure to compounds in male seminal fluid causes a brief and temporary increase in egg laying (Kalb et al. 1993). We expected that effects of mating on offspring quality might also be brief and temporary; therefore, we collected a single vial with eggs from each mother at maternal age 7, when mothers had been restricted from mating for either 5 days (low mating), 2 days (medium mating), or 0 days (high mating). During the middle of the interval when flies emerge as adults, 2 days after initial eclosion, we cleared the vials of all adults and 5 h later collected a single daughter from each vial (maternal age 19, daughter age 0). From the 182 mothers that made up trial 3, we collected a total of 172 daughters: 56, 60, and 56 from high-, medium-, and low-mating mothers, re-

spectively (eight mothers did not produce daughters that emerged during the 5-h collection interval or had died before laying eggs). Twenty-four hours later, the daughters from all of the maternal mating treatments were placed singly in vial-sized cages (as described before) with four 2–4-day-old virgin males chosen from a large pool of males. The daughters were transferred to new vials with their males every other day until death, and fresh 2–4-day-old virgin males were provided every 2 weeks.

Daughter age-specific fecundity and LRS were determined by counting all of the hatched pupal cases. Egg-to-adult viability of daughters was measured by counting the number of eggs that each daughter laid in every fourth 2-day interval. We recorded the age at death for each daughter. During the course of the experiment, 1.7% of all daughter flies (3/172) escaped or were lost as a result of handling errors. As with mothers, these flies were censored in the survival analysis and eliminated from the lifetime reproductive success analyses but were used in the egg viability and age-specific fecundity analyses for the intervals before they were lost.

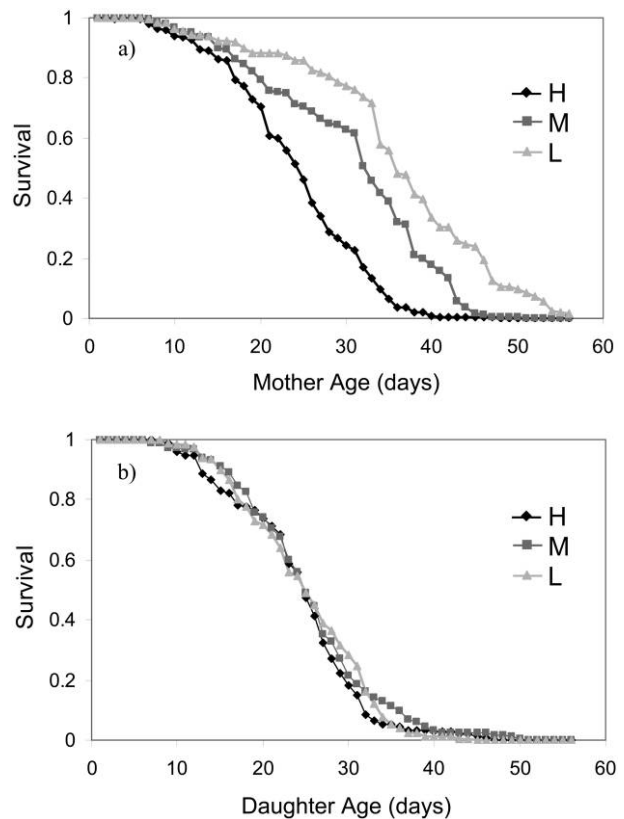


Figure 1: *a*, Survival curves for mothers from high-, medium-, and low-mating treatments. *b*, Survival curves for the daughters derived from the mothers from high-, medium-, and low-mating treatments.

Table 1: Ln likelihood analysis of the effects of maternal mating frequency on mortality intercept (*A*), rate (*B*), and leveling-off (*S*) parameters of the logistic mortality decomposition for the maternal generation

Comparison	<i>A</i>	<i>B</i>	<i>S</i>
High vs. low mating	159.90***	6.912**	.781
Medium vs. low mating	39.24***	3.033	3.235
High vs. medium mating	71.35***	.540	1.148

Note: The analysis examines mortality differences between pairs of treatments; χ^2 statistics are reported with $df = 1$ and statistical significance.

** $P < .01$.

*** $P < .001$.

Calculation of Fitness and Inclusive Fitness

Charlesworth (1970, 1994) showed that for a population with overlapping generations, the fitness (w) of a particular cohort i is described by

$$w_i = \sum_{x=0}^{\omega} e^{-mx} k_i(x), \quad (1)$$

where x is age of cohort i from adult emergence 0 through last reproduction ω and $k_i(x)$ is the age-specific product of survival and fecundity. We used this equation to estimate the fitness of high-, medium-, and low-mating mothers and their daughters for trial 3. We treated the roughly eight individuals of each mating treatment within each block as a cohort. Two values of m are most appropriate for estimates of fitness, LRS and λ . Evaluating w_i at $m = 0$ is equivalent to estimating fitness with LRS (Charlesworth 1994); λ is the value of m that can be found

implicitly by setting $w_i = 1$, using the Euler-Lotka equation (Lotka 1939). To understand how population growth rate assumptions influence fitness estimates, we evaluated the fitness of each cohort over the range of population growth rates commonly found in fruit fly population cage cultures ($m = -0.4$ to 0.4 ; Prout and McChesney 1985; for a similar approach, see Tatar and Promislow 1997). Evaluating fitness at $m = 0$ makes the implicit assumption that each offspring, regardless of when it is born, makes the same contribution to the next generation. Positive population growth rate assignments assume that early-age fecundity has the greatest contribution to fitness, while negative population growth rate assignments assume that late-age fecundity has the greatest contribution to fitness.

LRS and λ are inadequate measures of parental fitness when the survival and fecundity of parents influence the survival and fecundity of offspring (Hamilton 1964; Grafen 1984, 1988). When this is true, we need to measure inclusive fitness, the combination of an individual's total fitness, and the fitness the individual accrues by helping relatives. Charlesworth and Charnov (1981) and Oli (2003) calculate inclusive fitness from fitness differences between recipients and nonrecipients of the donor's aid, scaled by the degree of relatedness between the donor and the recipient. Thus, the nonrecipients are treated as controls, and the benefits of altruism are estimated from fitness differences between the recipients and the nonrecipients of the altruistic act. In addition, both models use the λ of the donor generation and the λ of the recipient generation to estimate inclusive fitness. (Oli [2003] ex-

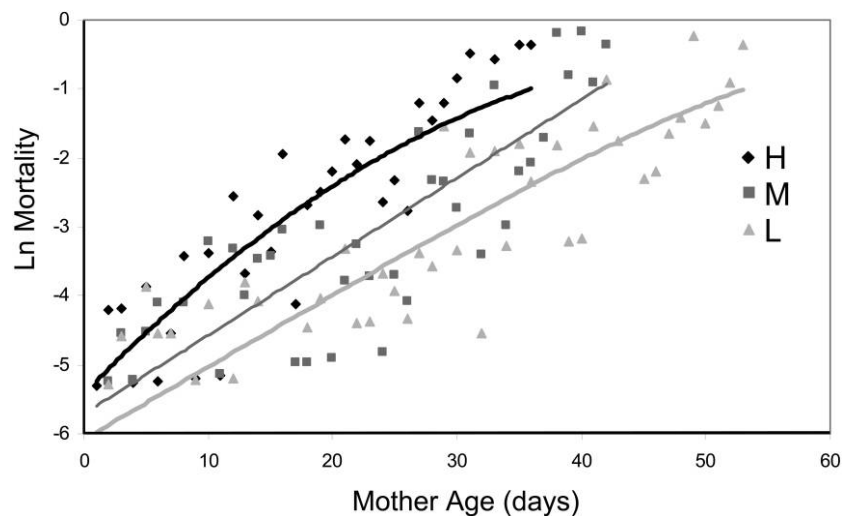


Figure 2: Age-specific mortality curves for mothers from high-, medium-, and low-mating treatments fit to a logistic model. High maternal mating frequency shows significantly higher background mortality (intercept) and rate of aging (slope).

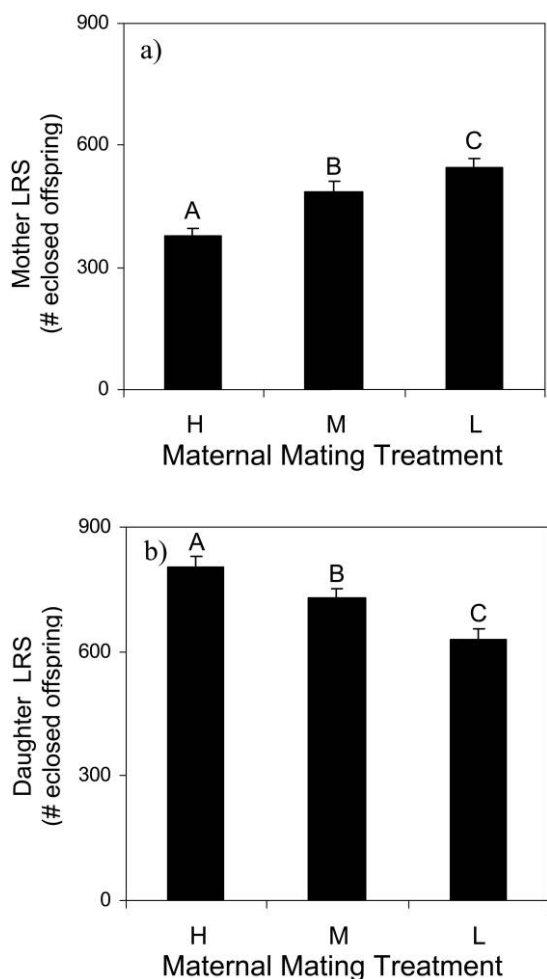


Figure 3: Lifetime reproductive success (LRS) of high-, medium-, and low-mating mothers (mean \pm SE; *a*) and their daughters (mean \pm SE; *b*) from trial 3. The different letters indicate that the means differ at $\alpha = 0.05$.

plicitly uses λ , and Charlesworth and Charnov [1981] use a related concept, reproductive value.)

However, the manner in which age is structured remains an issue that may bias measures of inclusive fitness. The models estimate inclusive fitness by combining fitness costs with respect to the age of the recipient generation and fitness benefits with respect to the age of the donor generation (Charlesworth and Charnov 1981; Oli 2003). In an expanding population, early-age fecundity has a disproportionate effect on the value of λ . Thus, the age at which the fitness costs and benefits of mating appear may have an impact on how altruism genes evolve. If the population is expanding and there is a time lag between the expression of fitness costs by the donor generation and the expression of fitness benefit by recipient generation,

then both models will underestimate the fitness costs and overestimate the fitness benefit of the altruistic act.

A different way of handling age structure across multiple generations is estimating the fitness benefits to the recipient with respect to the age of the donor, not the age of the recipient. This approach places cross-generational fitness trade-offs in an age-specific context that is appropriate when there is a time lag between the altruistic act and benefits to recipients. The time lag issue is relevant for this study because, in fruit flies, parental effects can occur only before egg laying and enhanced offspring fecundity can be expressed only at a later age (because daughters take several days to eclose).

Therefore, we define the inclusive fitness of a maternal cohort i by

$$w_{\text{inclusive}} = \sum_{x=0}^{\omega_i} e^{-mx} k_i(x) + \sum_{x=0}^{\omega_i} r_{ij} [e^{-mx} k_j(x) - e^{-mx} k_{\text{control}}(x)], \quad (2)$$

which combines the fitness of mothers with the proportion of daughter fitness that results from maternal mating (modified from Charlesworth and Charnov 1981; Oli 2003). For all terms, x is age of the maternal cohort i from adult emergence 0 through age of last reproduction of the mother ω_i and daughter cohorts ω_j ; m is the population growth rate of the maternal generation; and $k_i(x)$, $k_j(x)$, and $k_{\text{control}}(x)$ are the products of age-specific survival and fecundity of the mother, daughter, and control cohorts, respectively. Here, the $k_{\text{control}}(x)$ term is the age-specific product of survival and fecundity for daughters of low-mating mothers, and r_{ij} is the degree of relatedness between mothers and their daughters, $r_{ij} = 0.5$.

This metric calculates maternal fitness and combines that quantity with the amount of daughter fitness that results from maternal mating frequency. Clearly, not all of the fitness of daughters should be attributed to the mothers (Grafen 1984, 1988). This metric estimates the proportion of daughter fitness that directly results from maternal mating frequency by subtracting the fitness of daughters from high-mating mothers from the fitness of daughters of low-mating mothers (control). This quantity is then multiplied by 0.5 to account for the fact that only half of a daughter's genes come from her mother. The quantity is then scaled by m to account for the fitness consequences of exponential growth. For simplicity, we assign the same value of m for the control as for the high- and medium-mating treatment, although we recognize that this may slightly bias the results when the treatments have differences in age-specific survival or fecundity. This metric can generate estimates of inclusive fitness for high- and medium-mating mothers. Because low-mating mothers by definition do

Table 2: Repeated-measures ANOVA on effects of maternal mating frequency on age-specific changes in percent egg viability and fecundity (no. enclosed offspring) of mothers and daughters

Generation and variable	Age	Mating	Mating × age	Block
Mother:				
Egg viability	34.72 (4)***	.47 (2)	1.46 (7)	14.37 (2)***
Fecundity	20.82 (6)***	17.65 (2)***	1.57 (12)	2.63 (2)
Daughter:				
Egg viability	25.12 (4)***	.14 (2)	1.26 (8)	1.99 (7)
Fecundity	186.15 (19)***	36.35 (2)***	1.55 (38)*	2.09 (7)*

Note: The F statistics are reported with numerator degrees of freedom in parentheses and with statistical significance. The error degrees of freedom for the egg-to-adult viability and age-specific fecundity analyses were 268 and 435, respectively, for the maternal generation and 80 and 352, respectively, for the daughter generation.

* $P < .05$.

*** $P < .0001$.

not contribute any benefits of increased mating frequency to their daughters, the inclusive fitness of low-mating mothers is only the maternal fitness of low-mating mothers. We calculated the inclusive fitness of high- and medium-mating mothers and the fitness of low-mating mothers for each of the eight blocks in trial 3. To examine how population growth rate assumptions influence estimates of inclusive fitness, we evaluated inclusive fitness over a range of m that is common to normal fruit fly cultures ($m = -0.4$ to 0.4).

Statistical Analysis

The survival analyses were conducted with Proc PHreg (SAS 2003), which uses a maximum likelihood approach. The large sample sizes of the maternal generation allowed us to examine effects of mating frequency on aging. The mortality rate data fit a logistic curve, $\mu_x = Ae^{Bx}/[1 + (AS/B)(e^{Bx} - 1)]$, where A , B , and S are terms that describe age-independent mortality (intercept or background mortality), age-specific mortality (slope or rate of aging), and leveling off (the deceleration of mortality at late ages), respectively. We estimated the mortality intercept, rate, and leveling off for each mating treatment and tested for differences between pairs of mating treatments using WinModest (Pletcher 1999), a maximum likelihood model that accounts for censored data.

For the maternal generation, we tested for the effects of the maternal mating treatment on LRS, age-specific fecundity, and age-specific egg viability by using treatment means for each of the 24 blocks that comprised trials 1, 2, and 3. Block means were used in the analysis to reduce variation in the data. For the LRS data, we performed ANOVA with maternal mating treatment and block as fixed effects, and we used the Tukey multiple comparison test to compare differences between the mating treatments. To examine differences in age-specific fecundity, we performed repeated-measures ANOVA on age-specific fecun-

dity, with maternal mating treatment and block as fixed effects. Egg density did not differ among the maternal mating treatments ($F = 2.23$, $df = 2, 536$, $P > .11$). Egg density was tested as a covariate and was dropped from all analyses because it was not a significant source of variation. To statistically examine differences in mother, daughter, and inclusive fitness, we performed ANOVA on the values of w_i and $w_{\text{inclusive}}$ obtained from equations (1) and (2) at each value of population growth rate, with maternal mating treatment as a fixed effect. All analyses were done using PROC MIXED (SAS 2003) on untransformed data; both the data values and the residuals approximately fit model assumptions.

Results

The maternal mating treatment influenced the survival of mothers but not daughters. Mothers with the highest mating frequency had the lowest survival ($\chi^2 = 194.57$, $df = 2$, $P < .0001$; fig. 1a). The mortality analysis showed that mating frequency significantly influenced background mortality (mortality intercept) and the rate of aging (mortality rate) of mothers (table 1; fig. 2). The mortality intercepts of each mating treatment were significantly different from one another, with high-mating mothers having the highest mortality intercept and low-mating mothers having the lowest mortality intercept. The mortality rates of high- and low-mating mothers were significantly different, but there were no significant differences in the leveling-off term (table 1). There were no effects of maternal mating on daughter survival ($\chi^2 = 0.27$, $df = 2$, $P = .87$; fig. 1b).

Maternal mating frequency influenced both maternal fecundity and daughter fecundity, though in opposite ways for each generation. Increased maternal mating frequency reduced maternal LRS by 30% but increased daughter LRS by 28% (mothers: $F = 57.12$, $df = 2, 67$, $P < .0001$; daughters: $F = 12.31$, $df = 2, 21$, $P = .0003$; fig. 3). High-

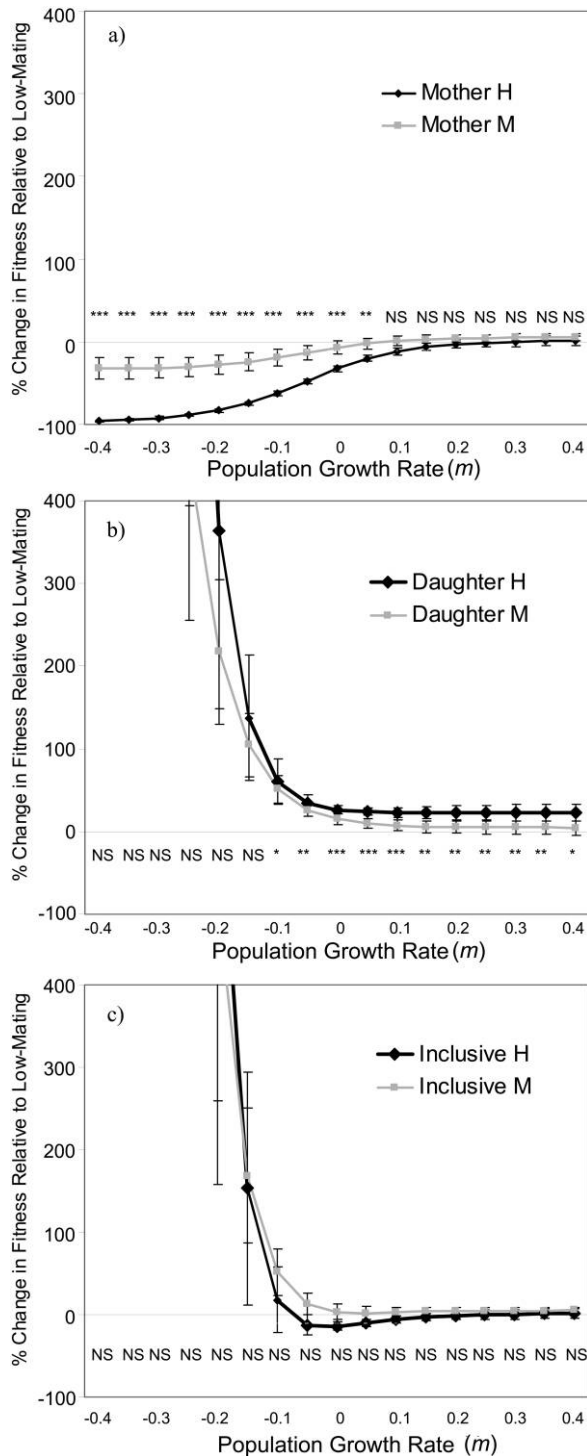


Figure 4: Effect of maternal mating on the fitness of mothers (a), the fitness of daughters (b), and inclusive fitness (c). The fitness of high- and medium-mating treatments are depicted relative to low-mating treatments. The effect of mating on fitness is reported for each value of m . Three asterisks, $P < .001$; two asterisks, $P < .01$; one asterisk, $P < .05$; NS, $P > .05$.

mating females had the lowest LRS, but their daughters had the highest LRS; medium-mating females had intermediate LRS, and their daughters had intermediate LRS; and low-mating females had the highest LRS, but their daughters had the lowest LRS (fig. 3). Similar statistically significant patterns were observed with respect to mother and daughter age-specific fecundity (table 2; fig. A1). In the maternal generation, the effect of maternal mating frequency on age-specific fecundity was consistent across maternal ages (no mating \times age interaction). However, high-mating mothers produced daughters with disproportionately greater early-age fecundity (table 2; fig. A1b). Maternal mating frequency did not influence egg-to-adult viability (table 2). Not surprisingly, age-specific fecundity and egg-to-adult viability decreased with female age (table 2; fig. A1).

Both mother and daughter fitness estimates were strongly influenced by the value of m that was assigned in the calculation of fitness. For the maternal generation, at all values of $m < 0.10$, increased maternal mating frequency reduced maternal fitness (table A1; fig. 4a). For the daughter generation, at all values of $m > -0.10$, increased maternal mating frequency significantly improved daughter fitness (table A1; fig. 4b). Note that at values of $m < -0.10$, increased maternal mating frequency substantially increased daughter fitness, but the effect was non-significant because of variation in late-age daughter fecundity, which, in a declining population, has a disproportionate contribution to fitness (fig. 4b). When the fitness cost of multiple mating to females and fitness benefits to daughters were placed in an inclusive fitness context, there was no significant effect of multiple mating on fitness at any value of m (table A1; fig. 4c).

Discussion

The results presented here reveal two patterns that are important to our understanding of how the mating frequency of females evolves in the face of substantial costs. First, increased maternal mating frequency improved the LRS and fitness of daughters. Second, multiple mating may not be as costly as is typically presumed. When the effects of maternal mating frequency on mother and daughter fitness were integrated in a cross-generational fitness approach, increased mating frequency was not costly to mothers at any assigned population growth rate.

With respect to the maternal generation, we found that multiple mating reduces female survival and LRS, which is a result similar to that of studies with comparable designs (Fowler and Partridge 1989; Brown et al. 2004). This study also revealed novel findings in the maternal generation. Multiple mating increased the age-independent mortality (intercept or background mortality) and the age-specific

mortality (slope or rate of aging) and decreased the age-specific fecundity of females. These results suggest that multiple mating accelerates the aging process by increasing the risk of death with age and by stimulating more rapid reproductive senescence. Previous work found that increased mating frequency decreases survival and longevity (Partridge and Andrews 1985; Fowler and Partridge 1989; Chapman et al. 1995; Brown et al. 2004), but this is the first study to confirm that mating frequency directly influences the aging process. Also, the effect of males on the background risk of death (mortality intercept) and rate of aging (mortality slope) are genetically correlated with male sperm competitive ability and male reproductive success (Civetta and Clark 2000; Sawby and Hughes 2001). Changes in patterns of mother mortality might therefore occur as a consequence of sperm competition. But it is also possible that changes in mother mortality could result from cross-generational fitness trade-offs between mothers and daughters. This hypothesis has been difficult to assess because, until now, the influence of maternal mating frequency on offspring fitness in fruit flies was unknown.

Several studies have found that mate choice, mate number, or mating frequency influences components of offspring fitness (Reynolds and Gross 1992; Tregenza and Wedell 1998; Newcomer et al. 1999; Evans and Magurran 2000; Konior et al. 2001; Tregenza et al. 2003; Schmoll et al. 2005). Our study is the first to show that mating frequency increases the LRS of offspring in a species where there are substantial costs of mating to females. On average, increased mating frequency improved the total fecundity of daughters by 28%. Our study implicates parental effects (Qvarnström and Price 2001; Kozielska et al. 2004) rather than genetic benefits (Jennions and Petrie 2000; Fedorka and Mousseau 2004) for the mechanism of how multiple mating affects offspring quality. The principal factors driving costs of mating to females are accessory gland proteins (Acps), which are produced by males and delivered to females in male seminal fluid (Chapman et al. 1995). Acps target tissues throughout the reproductive tract; some even enter the hemolymph of females and change female physiology (Wolfner 2002; Ram et al. 2005). Maternal exposure to Acps also increases the early fecundity and population growth rate of daughters (Priest et al. 2007). Thus, the effects of maternal multiple mating on daughter fecundity and fitness reported here may occur through Acp-induced parental effects.

There has been extensive debate about how to measure fitness in studies of sexual selection. Behavioral ecologists have primarily advocated LRS as the best estimate of fitness (Clutton-Brock 1988). However, others have endorsed calculating λ , the value of m that yields a fitness of 1 in the Euler-Lotka equation, to estimate fitness (Charlesworth 1994). There has also been debate about how to measure

inclusive fitness (Grafen 1984; Creel and Wasser 1994; Griffin and West 2002). Life-history and quantitative genetic theory indicate that methods that account for population growth rate should be used when population growth is nonzero and when parents influence the fitness of offspring (Charlesworth and Charnov 1981; Grafen 1984, 1988; Brommer et al. 2002; Oli 2003).

Because we evaluated our demographic data across a range of m , our approach provides insights into the importance of population growth rate for estimates of fitness. We found that the consequences of female reproductive strategies for fitness greatly depend on the value of m (see also Tatar and Promislow 1997; Head et al. 2005). If we focus only on the maternal generation, then at $m = 0$, increased mating frequency has significant fitness costs for females, but at highly positive values of m (which is closer to the value of λ in ideal culture conditions), increased mating frequency is not costly for females. Multiple mating does not reduce fitness at positive values of m because the early-life survival and fecundity of mothers from the different mating treatments is similar (see figs. 1a, A1a). The implication of this finding is that there is evidence for costs of mating to mothers in the Dahomey line of fruit flies when LRS is the fitness estimate but not when λ is used as the fitness estimate. When we incorporated the fitness of mothers that is expressed through their daughters in a measure of inclusive fitness, we found that at each assumed value of m , the costs of increased mating frequency to mothers were balanced by the benefits to daughters. Thus, when intergenerational effects are accounted for, there is no evidence that increased mating frequency has fitness costs to mothers at any assigned population growth rate.

In this analysis, a range of population growth rates was assigned to uncover how population growth rate assumptions affect estimates of inclusive fitness, not to identify the optimal female mating frequency. The fitness measures assume a constant environment and no density dependence, as is the case for LRS and λ fitness measures. These assumptions are not likely to be biologically realistic because in the field and in the lab, fruit fly population size and growth rate fluctuate over time (Prout and McChesney 1985). If we take this analysis further, the consequences of these assumptions could be evaluated using an invasion analysis that incorporates many generations of simulated population size fluctuations in conjunction with costs of mating that vary with resource availability (Chapman and Partridge 1996) and parental effects on offspring fitness.

A further extension of this cross-generational analysis could include an evaluation of how maternal mating frequency influences the fitness of sons. Previous studies have found negative correlations between fitness-related traits expressed in sons and daughters (Fedorka and Mousseau

2004; Pischedda and Chippindale 2006). If that were true here, the fitness benefits of mating-induced maternal effects that we found for daughters might be offset by fitness costs to sons. However, one recent study found that in fruit flies, the benefit of maternal mate exposure to sons did not compensate for fitness costs to mothers (Orteiza et al. 2005), suggesting that further study is needed to understand the general effect of maternal mating on offspring fitness.

Despite these limitations, our results provide new insights into the evolution of mating behavior. It is thought that multiple mating behaviors evolve either because of sexual conflict, where traits evolve that benefit males but harm females, or because of mutualism, where traits evolve that benefit both sexes (Pizzari and Snook 2003). In the first model, multiple mating would be expected to decrease the inclusive fitness of females, while in the second, multiple mating would increase the inclusive fitness of females. This study does not support either idea, as the inclusive fitness of females was neither significantly improved nor diminished by increased maternal mating frequency. We found that when females were restricted from free access to males, they responded by living longer and producing offspring with lower fecundity, which is more consistent with the social constraints hypothesis (Gowaty 1996, 1997) and with game theory than with sexual conflict (Chapman et al. 2003; Pizzari and Snook 2003). It is also possible that mating frequency does not evolve through sexual selection. Frequent mating increases the risk of death and accelerates the aging process of mothers, but it might be favored by selection if it changes resource allocation to offspring and increases the probability of producing highly fertile offspring. If there are genetic correlations between costs of multiple mating to mothers and fitness benefits to offspring, then mating frequency could evolve as an

indirect consequence of natural selection on inclusive fitness.

Our results have general implications for life-history evolution. We found that mothers with the highest rate of aging (age-specific mortality) produce offspring with the highest fitness. If there is a genetic basis to this physiological correlation, then it is possible that selection to optimize inclusive fitness can drive the evolution of shorter life and more rapid aging. This result might also help explain why we often find positive genetic correlations between survival and fecundity in natural and laboratory populations, a pattern that runs contrary to theoretical predictions (Reznick et al. 2000). Though certain lineages might produce parents that appear to be short lived and of low fecundity, the lineages could have high fitness and persist over time because they produce offspring that are well suited for the prevailing conditions in the population. Thus, an inclusive fitness theory of aging that accounts for population growth rate might help explain both the evolution of costly mating behaviors and the persistence of genetic variation in aging.

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APPENDIX

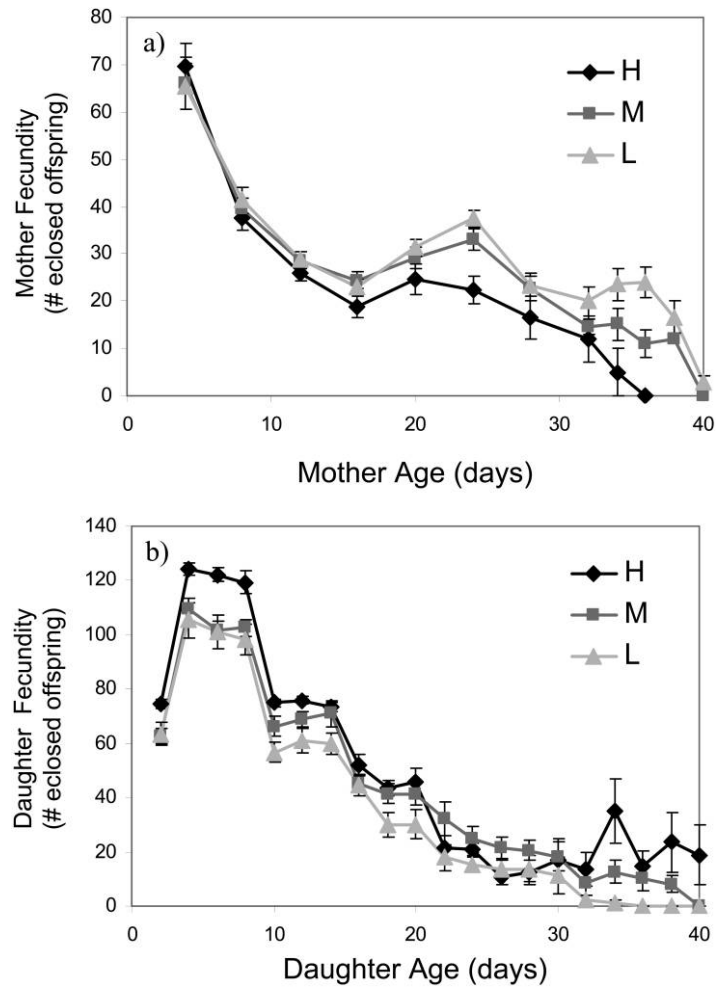


Figure A1: Age-specific fecundity (mean \pm SE) of high-, medium-, or low-mating (a) mothers measured in 2-day intervals every 4 days and (b) their daughters measured each 2-day interval.

Table A1: Effects of maternal mating frequency on mother, daughter, and inclusive fitness at different population growth rates (m)

m	Mother	Daughter	Inclusive
-.40	33.84***	1.41	1.39
-.35	39.33***	1.39	1.34
-.30	43.01***	1.37	1.29
-.25	44.34***	1.42	1.24
-.20	42.92***	1.60	1.17
-.15	38.94***	2.14	1.06
-.10	32.74***	3.53*	.99
-.05	24.60***	7.07**	2.09
0	15.47***	12.41***	3.22
.05	7.73**	12.79***	2.19
.10	3.29	10.65***	1.18
.15	1.45	8.94**	.70
.20	.80	7.78**	.52
.25	.57	6.98**	.46
.30	.49	6.40**	.44
.35	.45	5.97**	.44
.40	.44	5.64*	.44

Note: F statistics are reported with significance. For all analyses, $df = 2, 21$.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

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