Polyandry increases offspring viability and mother productivity but does not decrease mother survival in *Drosophila pseudoobscura*

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Polyandrous mating is common, but the benefits for females of polyandry remain controversial. To test whether mating with multiple males affects female fitness, we compared lifetime components of fitness of three experimental sets of *Drosophila pseudoobscura* females: monogamous females allowed to copulate one time (M_{OC}); monogamous females held with a male over her entire life and experiencing many copulations (M_{MC}); and polyandrous females with a different male over each day of their lives and also experiencing many copulations (P_{MC}). Consistent with previous studies in this species, females in treatments in which multiple copulations occurred, M_{MC} and P_{MC}, had offspring with significantly higher egg-to-adult survival (i.e., offspring viability) and higher numbers of adult offspring (i.e., productivity) than M_{OC} females, showing that multiple inseminations enhance offspring and mother fitness. In addition, although M_{MC} females laid significantly more eggs than polyandrous (P_{MC}) females, percent egg-to-adult survival and number of adult offspring were higher for P_{MC} than M_{MC} females, showing that polyandrous mating enhances the fitness of females more than multiply mating with only one male. Inconsistent with the cost of reproduction, lifespan was not significantly longer for M_{OC} females than for M_{MC} or P_{MC} females. To our knowledge, this is the first study to examine simultaneously in outbred WT *Drosophila pseudoobscura* the lifetime costs and benefits to females of polyandry, monogamy with a single copulation, and monogamy with repeat copulations.

fitness | mating system | monogamy | multiple mating

In nature, females in many taxa mate with more than one male (1, 2), and metaanalyses are consistent with the hypothesis that females gain fitness benefits from polyandry (3, 4). Females may mate with more than one male to (*i*) guarantee an adequate sperm supply (5); (*ii*) enhance access to nutritional or other resources (6); (*iii*) inhibit or reduce the effects of male harassment (7); or (*iv*) reduce the costs of inbreeding (8). Hypotheses *i*, *ii*, *iii*, and *iv* predict that polyandrous females lay more eggs and have more eclosing (i.e., adult) offspring than monogamous females (Table 1). Females also may multiply mate to enhance offspring health. The benefit to offspring health of polyandry may occur (*v*) when complementary parental immune alleles increase disease resistance in offspring (9, 10); (*vi*) when parental incompatibilities in offspring resulting from endosymbionts or other factors are reduced (11); or (*vii*) when females gain access to good genes from population-wide "best" or "near-best" males (12). Hypotheses *v*, *vi*, and *vii* predict that polyandrous females have healthier offspring so that more survive to reproductive age than do monogamous females (Table 1). Finally, females also may mate with multiple males as (*viii*) a correlated response to selection on males to mate with more than one female (13). The hypothesis that polyandry is not a result of selection on females but rather a correlated response to selection on males for multiple mating predicts no fitness benefits for polyandrous females compared with monogamous females with multiple or single copulations (Table 1).

Interest of evolutionary biologists in polyandry goes back to Bateman (14), who reported that increased numbers of mates enhanced male fitness, but not female fitness in *Drosophila melanogaster*, an observation now called the Bateman principle (15–17) or Bateman hypothesis (18). In *D. melanogaster*, the species Bateman studied, modern investigation has revealed that females are polyandrous, which is often interpreted as a way for females to avoid sperm limitation (4, 18–20), which we define here as insufficient sperm to fertilize all a female's eggs as well as possible inviability of the sperm she may receive. In addition, experimental study of *D. melanogaster* (21) failed to reveal a benefit for polyandry over the benefit of copulating more than one time with the same male.

In *Drosophila pseudoobscura*, sperm limitation favors females who copulate more than once. This conclusion came from an experimental study in which females who copulated with the same male repeatedly compared with females who copulated only one time had a significantly higher number of offspring at the age of eclosion (5), a likely result of sperm limitation (because females do not receive enough sperm and/or that the sperm she does receive are inviable) in single mated females. Previous mate choice studies in *D. pseudoobscura* (22–24) showed that females and males paired with their individually preferred partners had offspring with higher egg-to-adult survival, higher numbers of eclosed offspring (i.e., productivity), and higher net reproductive success than individuals constrained to pairings with their non-preferred partners. Because mating with preferred partners in nature may be constrained by dispersal limitation or social competition or sexual coercion (25), the constrained female hypothesis (7, 26) predicts that a benefit of polyandry beyond that from multiply mating with the same male is enhanced offspring health. Like *D. melanogaster*, *D. pseudoobscura* mate multiply in nature (27–29). Thus, we tested whether a benefit of polyandry is enhanced offspring viability. We designed the current experiment to evaluate whether female *D. pseudoobscura* have a benefit from polyandry beyond guarding against sperm limitation.

The experiment had three treatments: (*i*) females allowed only a single day's exposure to a single male, i.e., monogamy with one copulation (M_{OC}); (*ii*) females allowed constant access to the same male throughout the experimental duration, i.e., monogamy with multiple copulations (M_{MC}); and (*iii*) females allowed access to a different male, but only one per day, on each day of the experiment, i.e., polyandry (P_{MC}).

We tested the prediction of the sperm limitation hypothesis that (*i*) multiple mating with the same male enhances female fitness over that expected from a single copulation. We also tested the

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Table 1. Benefits and costs to polyandry hypotheses predict variation in different components of fitness and the direction of effect in experimental treatments

Polyandry hypotheses/tested component of fitness	Predicted	Observed
Guards against sperm limitation (not enough sperm or inviable sperm)		
Eggs laid	$M_{MC} > M_{OC}$	$M_{MC} = M_{OC}$
Eclosed offspring	$M_{MC} > M_{OC}$	$M_{MC} > M_{OC}$
Egg-to-adult survival	$M_{MC} > M_{OC}$	$M_{MC} > M_{OC}$
Enhances offspring survival		
Eclosed offspring	$P_{MC} > M_{MC}$	$P_{MC} > M_{MC}$
Egg-to-adult survival	$P_{MC} > M_{MC}$	$P_{MC} > M_{MC}$
Male peptides manipulate female egg-laying rate		
Eggs laid	$P_{MC} > M_{MC} > M_{OC}$	$P_{MC} < M_{MC} = M_{OC}$
Assuming there are no flexible adjustments of male peptides in ejaculates that could harm females		
Female lifespan	$P_{MC} < M_{MC} < M_{OC}$	$P_{MC} = M_{MC} = M_{OC}$
Assuming there is flexible adjustment of male peptides in ejaculates that could harm females		
Female lifespan	$P_{MC} > M_{MC} = M_{OC}$	$P_{MC} = M_{MC} = M_{OC}$
Ejaculate contributions nourish zygotes and/or females		
Eclosed offspring	$P_{MC} \geq M_{MC}$	$P_{MC} > M_{MC}$
Lifespan of females	$P_{MC} \geq M_{MC}$	$P_{MC} = M_{MC}$
Correlated response to selection on males to mate multiply		
Eggs laid	$P_{MC} = M_{MC}$	$P_{MC} < M_{MC}$
Eclosed offspring	$P_{MC} = M_{MC}$	$P_{MC} > M_{MC}$
Egg-to-adult survival	$P_{MC} = M_{MC}$	$P_{MC} > M_{MC}$

Experimental treatments included (*i*) females with access to one male during only 1 d of their lives, i.e., monogamous females with one copulation M_{OC}; (*ii*) females with continuous access to the same male, i.e., monogamous females with multiple copulations M_{MC}; and (*iii*) polyandrous females with access to a different male on each day of the experiment, i.e., polyandrous females with multiple copulations, P_{MC}.

fitness predictions of subhypotheses about the effects of male copulatory contributions to females. Under the "male peptides manipulate female egg-laying rate" hypothesis, peptides in male ejaculates are expected to increase female egg-laying and decrease female survival rates, leading to the prediction that M_{MC} and P_{MC} females lay more eggs than M_{OC} females and that M_{MC} and P_{MC} females die faster than M_{OC} females. Under the assumption that harmful male peptides are flexibly expressed depending on whether males and females are in preferred or nonpreferred matings (23, 30), those females with more options,
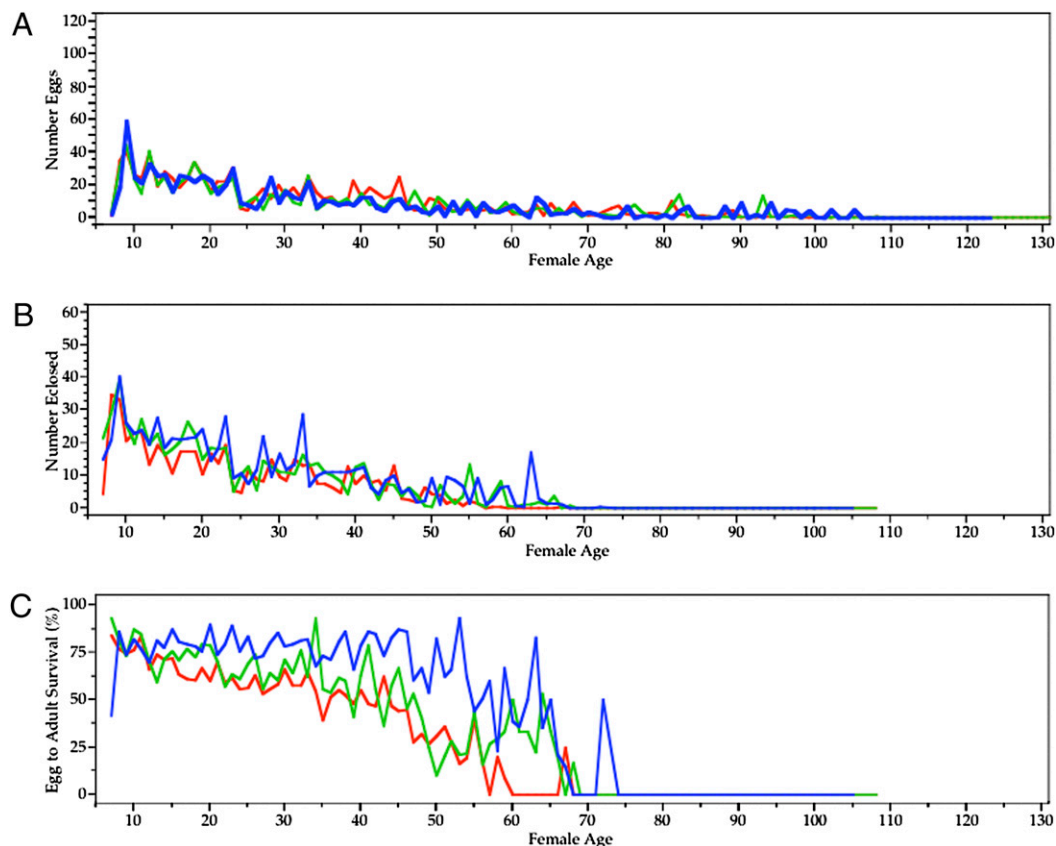

Fig. 1. Time course of (*A*) number of eggs laid, (*B*) number of offspring eclosed as adults, and (*C*) percent egg-to-adult survival for P_{MC} (blue), M_{MC} (green), and M_{OC} (red) females. The mean numbers of eggs laid per female per day of life were 7.4 ± 9.7 (SD) for M_{OC} females, 6.7 ± 8.9 for M_{MC} females, and 7.3 ± 9.4 for P_{MC} females. (*B*) The mean number of offspring eclosed (productivity) was 5.6 ± 7.7 for M_{OC} females, 7.2 ± 8.8 for M_{MC} females, and 8.5 ± 9.2 for P_{MC} females. The mean egg-to-adult viability percentages were 28.0 ± 29.3 for M_{OC} females, 35.8 ± 31.5 for M_{MC} females, and 49.6 ± 35.2 for P_{MC} females.

the P_{MC} females, would more likely avoid harmful male peptides than those females with fewer options (31). This predicts that P_{MC} females sometimes live longer than M_{MC} or M_{OC} females. The difference turns on the assumption that males can up-regulate harmful peptides flexibly. If males do not flexibly express harmful peptides, the constant harm prediction would be more likely. If males deliver resources to females useful in oogenesis (6) or otherwise, multiple copulations with the same male or mating with several different males should enhance the number of offspring eclosing; thus, the hypothesis of male-delivered resources predicts that M_{MC} and P_{MC} females have more adult offspring and perhaps live longer than M_{OC} females. Finally, we tested whether polyandry enhances egg-to-adult survival over that achieved via monogamy when monogamous females received ejaculates on more than 1 d (i.e., $P_{MC} > M_{MC}$). Because the experimental design involved holding females in treatments until they died, we were also able to test whether (*i*) females exposed to the sperm and seminal peptides of multiple males (P_{MC}) die faster than those exposed to the sperm and peptides of only one male (M_{MC}) and (*ii*) whether females that lay the most eggs die faster than females that lay the least.

Results

The time course for female and offspring fitness (number of eggs laid, number of eclosed offspring, and percent egg-to-adult survival) in the three treatments is shown in Fig. 1.

As a direct test of the sperm limitation hypothesis, we examined fitness variation between M_{OC} and M_{MC} females. There was no significant difference in the number of eggs laid by M_{OC} and M_{MC} females (Fig. 2*A*). However, M_{MC} females produced 1.6 more adult offspring per day than M_{OC} females, a significant difference (Fig. 2*B*), as a result of the mean 5% per day increase in egg-to-adult survival of M_{MC} compared with M_{OC} females (Fig. 2*C*). Because M_{MC} females copulated more than one time, but always with the same male, this treatment eliminated polyandry as a potential explanation for the observed fitness benefits. We concluded, as others have (5), that female *D. pseudoosbcura* who copulate on only 1 d do not get enough sperm or enough viable sperm to fertilize a lifetime of egg production.

To test hypotheses that predict offspring viability benefits of polyandry, we asked whether P_{MC} females produced more offspring with higher viability than M_{MC} females. The experimental design compared M_{MC} females, confined for life with the same male, with P_{MC} females, also with lifetime male access, but with a different male on each day. By matching the age and mating experience of all males in the M_{MC} and P_{MC} treatments, we controlled for possible sperm limitation that could result when females were mated to older or exhausted males and which could result in lower fitness of M_{MC} than P_{MC} females.

M_{MC} females laid more eggs than P_{MC} females (Fig. 3*A*); on average M_{MC} females laid two more eggs per day than P_{MC} females (Fig. 3*A*). Despite laying fewer eggs than M_{MC} females, P_{MC} females produced significantly more adult offspring than
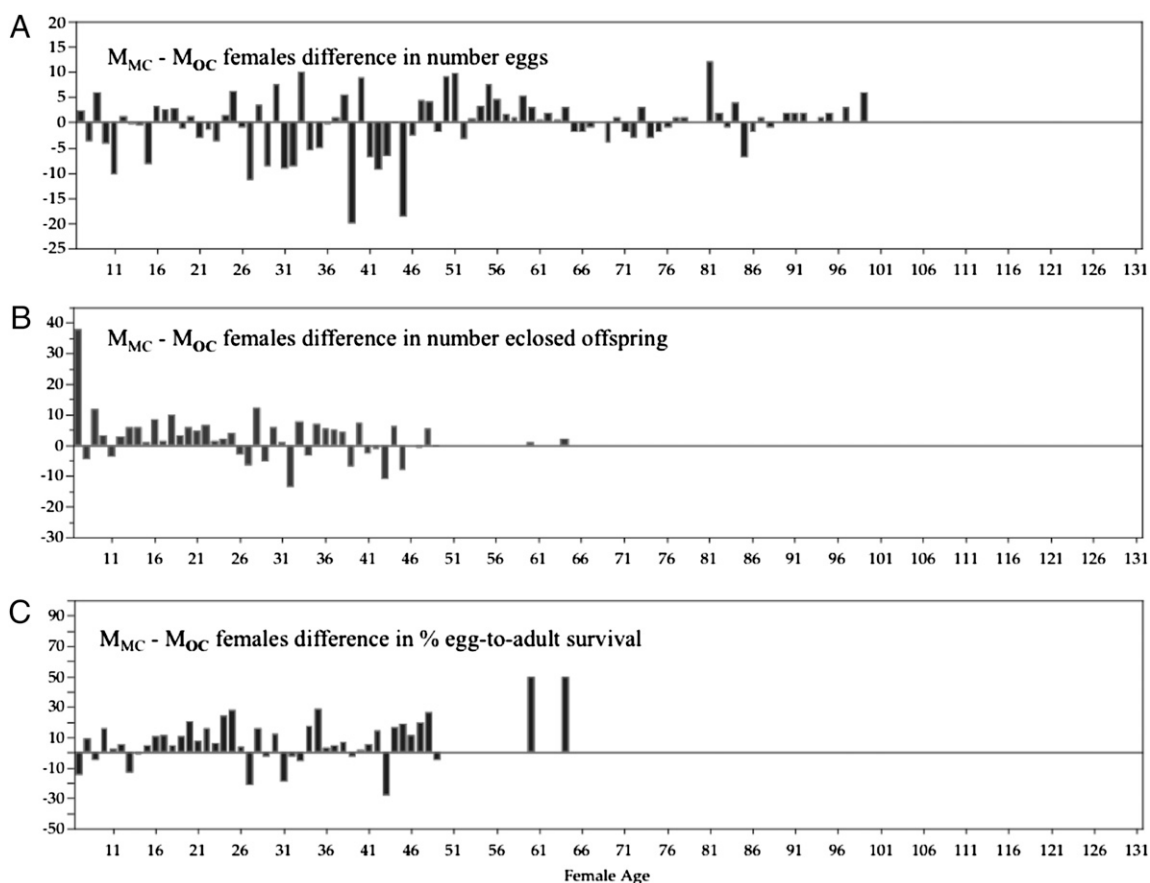

Fig. 2. Difference scores ($M_{MC} - M_{OC}$ females) for number of eggs laid (*A*), number of offspring eclosed as adults (*B*), and egg-to-adult survival percentage (*C*). The difference scores over days of female life were independent, and paired comparisons testing for deviations from zero showed that number of eggs laid (Wilcoxon signed-rank test, 62.0; $P > |t| < 0.792$) was not statistically significantly different between M_{MC} and M_{OC} females. However, the number of offspring eclosed as adults (Wilcoxon signed-rank test, 199.5; $P > |t| < 0.019$), and the egg-to-adult survival percentage (Wilcoxon signed-rank test, 295.5; $P > |t| < 0.0001$) were significantly higher in M_{MC} than in M_{OC} females.
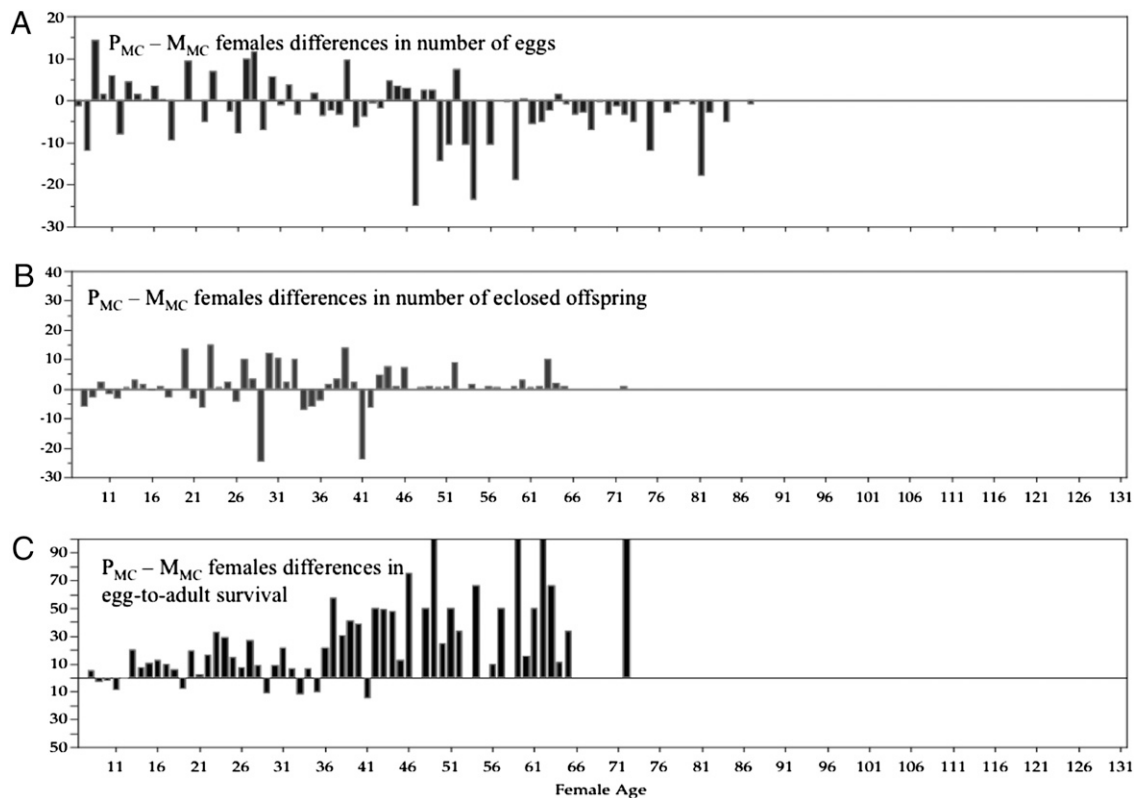
Fig. 3. Difference scores ($P_{MC} - M_{MC}$ females) for number of eggs laid (*A*), number of offspring eclosed as adults (*B*), and egg-to-adult survival percentage (*C*). The difference scores over days of female life were independent and paired comparisons testing for deviations from zero showed that number of eggs laid (Wilcoxon signed-rank test, −745.5; $P > |t| < 0.001$), number of offspring eclosed as adults (Wilcoxon signed-rank test, 247; $P > |t| < 0.001$), and egg-to-adult survival (Wilcoxon signed-rank test, 654; $P > |t| < 0.001$) were significantly different between M_{MC} and P_{MC} females.

M_{MC} females, i.e., on average P_{MC} females produced one more offspring per day than M_{MC} females (Fig. 3*B*). This was a result of the significantly higher egg-to-adult survival from P_{MC} than M_{MC} females (Fig. 3*C*). On average there was a 24% per day difference in egg-to-adult survival between P_{MC} and M_{MC} females. Because major differences in percent egg-to-adult survival accumulated after the median age of death of P_{MC} and M_{MC} females (Fig. 4), we retested for differences using only data on treatment females up to d 37. Even with this restriction, egg-to-adult survival was 10% per day higher for P_{MC} than M_{MC} females. The difference in fitness between M_{MC} and P_{MC} females resulting from enhanced egg-to-adult survival is a benefit of polyandry, which translates over time into significantly higher numbers of offspring produced for P_{MC} than M_{MC} females.

To examine the costs to females of polyandry, we compared the longevity of females in the three treatments. Longevity of females did not differ significantly by treatment (Fig. 4*A*). The length of life after production of their last surviving offspring did differ significantly (Fig. 4*B*), probably because M_{OC} females quit producing eclosing offspring sooner than M_{MC} and P_{MC} females.

There were no significant differences in postreproductive lifespan for females exposed to the sperm of more than one male (P_{MC}) compared with females that were repeatedly exposed to the sperm of a single male (M_{MC}). Lifespan similarities of P_{MC} and M_{MC} females do not indicate costs to females of polyandry.

Discussion

We conclude that there are few or no apparent costs and at least two fitness benefits of polyandry for females (1). Polyandrous mating reduces the likelihood that female reproductive success is affected by sperm limitation, which we defined as either not

enough sperm or inviable sperm. Our methods were not designed to discern between these two types of sperm limitation (2). Polyandrous mating enhances egg-to-adult survival and the number of adult offspring, observations consistent with hypotheses of indirect benefits for polyandrous females.

Other benefits of polyandry seem unlikely in *D. pseudoobscura* females. We eliminated or at least reduced the opportunity for male harassment of females to induce polyandrous mating because we never exposed females to more than one male at a time. Forced copulation of teneral females sometimes occurs in *D. pseudoobscura* (25). However, in our experiment we used only sexually mature virgin females, eliminating the known period of greatest vulnerability of females to forced copulation. There is no postzygotic parental care in *D. pseudoobscura* and we used populations free of *Wolbachia* and other endosymbionts (*Materials and Methods*) that produce breeding incompatibilities between parents in other species; thus, we do not think our results can be explained by reduction of breeding incompatibilities. In addition, because fitness differences between P_{MC} and M_{MC} occurred (Table 1)—that is, because benefits of polyandry were obvious whereas costs of polyandry were not—there is no support from this study for the hypothesis that polyandry is a correlated response to selection on males for polygyny.

We conclude that variation in sperm numbers or sperm viability explains the differences in fitness between M_{OC} and M_{MC} females (5), and that enhanced offspring survival explains the benefit that P_{MC} females have compared with M_{MC} females. Male *D. pseudoobscura* ejaculate many very small sperm, in fact, more sperm than in any other known *Drosophila* species (32), and males may contribute nutritional elements to females or their offspring through their ejaculates (6). Thus, the mecha-
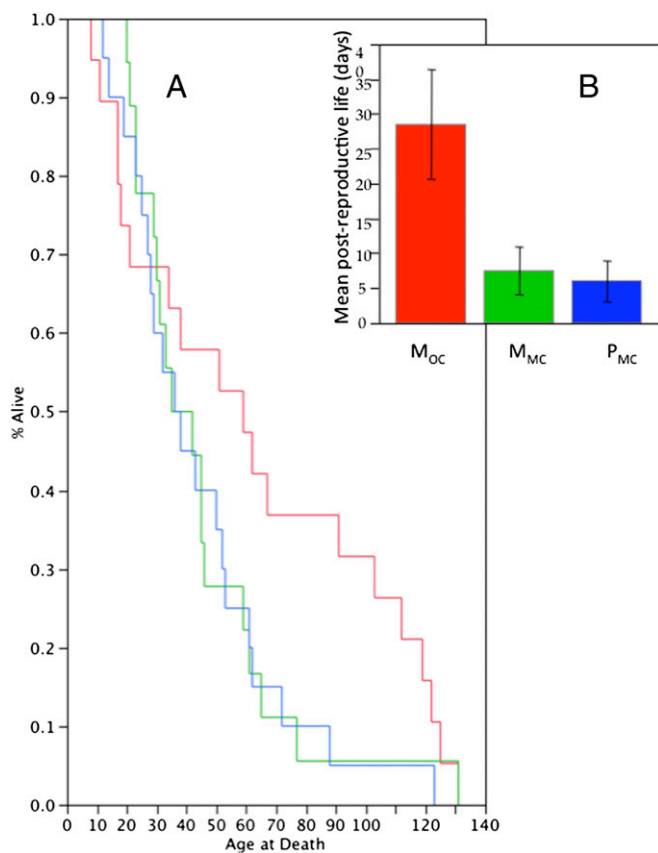
Fig. 4. (*A*) Lifespan and (*B*) days of postreproductive life of M_{OC} (red lines), M_{MC} (green line), and P_{MC} (blue line) females. The mean age of females when they produced their last eclosing offspring was 34.9 ± 4.1 d for M_{OC} females; 37.8 ± 4.2 d for M_{MC} females; and 38.6 ± 4 d for P_{MC} females ($F = 0.2042$; df = 56; $P > F = 0.8159$). The mean $\pm$ SE ages of death were 63.5 ± 10.15 d for M_{OC} females, 45.3 ± 6.35 for M_{MC} females, and 44.4 ± 6.1 for P_{MC} females ($F = 1.3579$; df = 59; $P = 0.2654$). Kaplan-Meier survival analysis for M_{OC} (red), M_{MC} (green) and P (blue) females indicated no significant differences in lifespan (log-rank $\chi^2 = 2.9505$, 2 df, $P < 0.2287$; Wilcoxon $\chi^2 = 1.069$, 2 df, $P < 0.5859$). *Inset*: Differences in mean number of days until death after production of their last surviving offspring. There were significant differences in days of postreproductive life between M_{OC} and P_{MC} females ($P < 0.0034$) and between M_{OC} and M_{MC} females ($P < 0.0072$), but not between M_{MC} and P_{MC} females ($P < 0.841$). There were no significant differences in lifespan if the experiment was considered ended by d 30, d 50, or d 70; i.e., there were no early deficits in survival of M_{OC} females and no significant survival benefit for M_{OC} females before the cessation of reproduction by all three types of females.

nisms that may mediate the offspring viability benefit of polyandry include (*i*) multiple and variable doses of any paternally derived nutritional contributions to females or zygotes, (*ii*) complementary parental alleles coding for offspring immune function, or (*iii*) best male genes.

The experiment revealed no costs of polyandry for females. Because there are no significant lifespan differences between females in the three experimental treatments, particularly between M_{MC} and P_{MC} females, polyandry appears to levy no additional costs over the costs to females of lifetime exposure to males.

The experimental results raise a number of questions about polyandry in the field and in the laboratory. How many sires are enough to ensure females have adequate sperm numbers or enough viable sperm? Do aged or more experienced males exhaust their abilities to produce viable sperm? What are the costs to males mated to M_{MC} females of compensatory increases in ejaculate size such as those that occur in *D. pseudoobscura* (30) when males are paired with females they do not prefer? Similarly, what are the costs

to females in M_{MC} treatments, when they are paired for life with partners they prefer or do not prefer? How do polyandrous females allocate sperm to eggs over their lifetime given that they store sperm, as *D. pseudoobscura* females do, and given that the quality of males whom they mate probably varies? Do females favor the sperm of some males over others when they have constant exposure to potential mates? Is the benefit of enhanced offspring viability a result of good genes from best males, parental dissimilarities at immune coding loci, or both? Do females compete over access to the number of mates or the quality of mates, and how confounded are numbers and quality of mates in species with sperm storage such as *D. pseudoobscura*? Given that polyandry is so common in nature, we wonder if polygynous mating in males might not be a result of correlated male trait evolution to polyandry in females. Alternatively, given that both male and female *D. pseudoobscura* assess potential mates before accepting or rejecting them, and given that both sexes have higher offspring viability when reproducing with mates they prefer (22), it is likely that similar selective pressures may act on the sexes to favor polyandrous females and polygynous males. More experiments, particularly between-species comparative experiments designed to evaluate the effects of polyandry in demographic context over the lifespan of females and males, and that take into account multiple fitness components, would be useful in resolving remaining debates about the benefits of polyandry.

Materials and Methods

W.W.A. collected *D. pseudoobscura* in Mesa Verde National Park (Colorado) in 1996 and maintained them in isofemale lines until July 1997, when a population cage was set up using eight isofemale lines. The lines we tested were free of *Wolbachia*, a vertically transmitted endosymbiont of insects, including some populations of *D. pseudoobscura* that are known to increase the remating rate of female *D. pseudoobscura* (33). Twenty cups containing cornmeal-yeast-molasses medium were placed in the cage, and every other day, three times a week, a new cup replaced one of the old cups. Approximately 80 generations later, we used flies from this cage for the experiment. We did this experiment from January through May 2004. We placed four fresh bottles in the cage for 1 wk, and then removed them and divided the food containing eggs and larvae into three new bottles. From these bottles, we collected virgin flies every 8 h, twice per day, and sexed them under CO_2. We maintained up to 10 same-sex flies per vial. We randomly picked flies from the same-sex vials for inclusion in this experiment, when flies were 7 d old.

Experimental Treatments. The experiment had three treatments: (*i*) monogamous females with access to a male for up to 24 h, who copulated only one time (M_{OC}); (*ii*) monogamous females each with a single male until she died, and who copulated many times (M_{MC}); and (*iii*) polyandrous females, who had access to one male per day, but a new male each day, so that P_{MC} females also copulated many times.

For each of the treatments, we made random pairings from 10 vials containing 7-d-old virgin females and 10 vials containing 7-d-old virgin males. We labeled vials as 0 through 9. Using a random number generator (0–9), we randomly picked flies from the vials to make 20 pairs for each treatment, and named them A1–A20 (M_{OC}), B1–B20 (M_{MC}), and C1–C20 (P_{MC}).

For the M_{OC} treatment, 24 h after pairings, we moved the females to new vials and discarded the males. For the M_{MC} treatment, 24 h after pairing and each day afterward, we moved the female and the male to a new food vial. For the P_{MC} treatment, 24 h after the initial pairing and on each day afterward, we replaced the male with another male the same age and with similar exposure to females. For example, the vial with female C1 received the male that was with female C20 the day before. The vial with female C2 received the male that was with female C1 the day before, and so on. We kept a bottle with extra males and females the same ages as the flies in this experiment, and replaced males that died with males from the reserve jar.

We observed M_{OC} females on their only day of exposure to males until copulation occurred (usually within 1 h), and scanned for copulations by M_{MC} and P_{MC} females after moving them daily between vials. We often saw M_{MC} and P_{MC} females copulating, but we did not count copulations.

We held 20 rubber-banded sets of vials with experimental classes of females (e.g., A1-B1-C1) together in incubators for the duration of the experiment to control for environmental effects on components of fitness.

Components of Fitness. We counted the number of eggs laid under a dissecting microscope after transferring females (and their mates) to new vials. After larvae had begun to eclose, we counted the number of emerging adult flies from each vial for 5 d. We noted the day on which each female died. We calculated percent egg-to-adult survival as number eclosed/number of eggs laid × 100.

Statistics. Based on the Fisher κ statistic, we found no evidence for temporal autocorrelation of difference scores ($M_{OC} - M_{MC}$ and $P_{MC} - M_{MC}$) for any component of fitness. Thus, for both sets of difference scores ($M_{OC} - M_{MC}$ and $P_{MC} - M_{MC}$), we used Wilcoxon signed-rank tests to evaluate the hypothesis that the distribution of difference scores was equal to 0.

We used univariate Kaplan-Meier statistics to test for differences across treatments in female lifespan. After testing for normality, we used ANOVA to test for differences between treatments in mean ages of death, ages that females laid their last eggs, the ages they produced their last eclosing offspring, and postreproductive lifespan (the number of days a female survived after producing the last egg that eclosed). We tested the distribution over days of the difference scores in number of eggs laid, number of eclosing adult offspring, and percent egg-to-adult survival for M_{OC} and M_{MC} females and for M_{MC} and P_{MC} females separately. The a priori significance level was set at less than 0.05.

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