Mating rate and fitness in female bean weevils

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Females of most animal taxa mate with several males during their lifespan. Yet our understanding of the ultimate causes of polyandry is incomplete. For example, it is not clear if and in what sense female mating rates are optimal. Most female insects are thought to maximize their fitness by mating at an intermediate rate, but it has been suggested that two alternative fitness peaks may be observed if multiple costs and benefits interact in determining the relationship between mating rate and fitness. We studied the relationship between female fitness and mating rate in the bean weevil, Callosobruchus maculatus (Coleoptera: Bruchidae), a species in which several distinct direct effects of mating to females have been reported. Our results show that female fitness, measured as lifetime offspring production, is lowest at an intermediate mating rate. We suggest that this pattern is the result of multiple direct benefits to mating (e.g., sperm replenishment and hydration/nutrition effects) in combination with significant direct costs to mating (e.g., injury from male genitalia). Females mating at low rates may efficiently minimize the costs of mating, whereas females mating at high rates instead may maximize the benefits of mating. If common, the existence of bimodal relationships between female mating rate and fitness may help explain the large intra- and interspecific variation in the degree of polyandry often seen in insects. *Key words:* Bruchidae, multiple mating, nuptial gifts, polyandry, sexual selection, sperm competition. *[Behav Ecol]*

Females of the vast majority of animals are polyandrous, such that they reproduce with several different males during their life. This fact sets the stage for a large variance in male reproductive success and is hence one of the corner stones of sexual selection theory. Understanding the evolution of female polyandry is therefore key to our understanding of sexual selection. Although many of the direct and indirect costs and benefits of mating to females are now well documented (for reviews, see Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000), our understanding of the evolution of polyandry is incomplete. For example, it is still not clear if and in what sense female mating rates are optimal (Arnqvist and Nilsson, 2000), or whether females are constrained (Wiklund et al., 2001) or manipulated by males (Holland and Rice, 1998) into mating suboptimally.

When the marginal benefits of remating to females exceeds the costs, female fitness should simply increase monotonously with elevated mating rate (Arnqvist and Nilsson, 2000; Shuster and Wade, 2003), and we expect females to be strongly polyandrous. This may, for example, occur in mating systems in which males transfer large and nutritious nuptial gifts to females at mating (see Vahed, 1998; Wiklund et al., 2001) and/or in which the costs of mating are insignificant. However, in the majority of animal species, mating is associated with sizable direct costs to females (see Arnqvist, 1989; Chapman et al., 1993, 1998; Daly, 1978; Hurst et al., 1995; Siva-Jothy et al., 1998; Thornhill and Alcock, 1983; Watson et al., 1998; Wing, 1988). Such costs may be additive (Arnqvist and Nilsson, 2000) or multiplicative (Johnstone and Keller, 2000). When costs are significant and there are diminishing rewards of mating multiply, females are predicted to maximize fitness by mating at some intermediate rate (Figure 1) (Arnqvist and Nilsson, 2000; Gavrilets et al., 2001;

Holland and Rice, 1998; Parker, 1979). Although this may often be the case, there are situations in which we expect the relationship between female fitness and mating rate to be more complex. Wedell et al. (2002) recently suggested that additional direct benefits that interact with other costs and benefits of polyandry could lead to more complex relationships. For example, if mating brings additional direct benefits that increase at a low but exponential rate with elevated mating rate, we instead expect two distinct female fitness peaks (Figure 1). Females mating at a low rate would then be efficient cost-minimizers, whereas female mating at a very high rate would be benefit-maximizers (see Wedell et al., 2002). Any mating factors that need to accumulate before positive effects are realized, such as hydration or certain nutrition benefits, could contribute to such a pattern.

In recent years, the bean weevil, Callosobruchus maculatus (Bruchidae), has become a model system for the study of sperm competition, male-female mating interactions, and sexual conflict. Females mate multiply, and a few experiments have addressed various aspects of the economics of mating interactions. Crudgington and Siva-Jothy (2000) showed that females are injured internally by the spiny genitalia of males during copulation, and that the degree of injury suffered by females increases in an additive fashion over successive matings. There are also good reasons to believe that males transfer seminal substances with additive and toxic effects in females, as such compounds have been documented in a closely related Bruchid species (Das et al., 1980). Females can store viable sperm from a single mating for their entire adult life in the laboratory (Crudgington, 2001), and these facts collectively suggest that we might expect a single female fitness optima at a very low mating rate. However, male bean weevils also transfer a substantial ejaculate (up to 7.9% of their body weight; Savalli and Fox, 1998), and Fox (1993a,b) has suggested that receipt of the ejaculate may in itself actually benefit females. Studies of the effects of multiple mating on female performance in this species have, interestingly enough, reached partly incongruent results. For example, Crudgington (2001) found that multiple mating
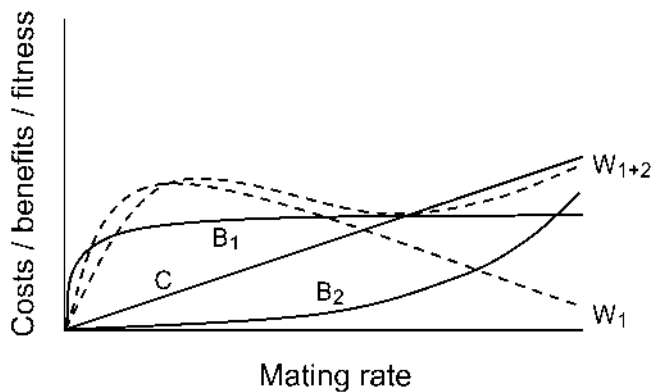
Figure 1
In mating systems in which the direct costs of mating are significant and approximately additive (C), females will maximize fitness by mating at some intermediate rate (W_1) if there are diminishing rewards of a single form of direct benefit of mating (B_1; e.g., replenishment of sperm supplies). However, if there are additional forms of benefits to females that increase at a low but accelerating rate (B_2), two distinct fitness peaks will instead be predicted: females maximize fitness by mating either at a low or a high rate (W_{1+2}).

reduces female lifespan, whereas Fox (1993b) found the exact opposite. Similarly, although several studies have found that multiple mating increases total female lifetime egg production (Crudgington, 2001; Savalli and Fox, 1999; Wilson et al., 1999), others have found no such effect (Fox, 1993b). Further, although Savalli and Fox (1999) found that females lay many eggs after the receipt of large amounts of ejaculate, Eady and Brown (2000) found the opposite. Thus, our understanding of the effects of multiple mating in *C. maculatus* is clearly incomplete. It has been suggested that these inconsistencies may result from females experiencing several interacting direct costs/benefits and/or from genetic differences between populations of this beetle (Crudgington, 2001; Eady and Brown, 2000).

Here, we report an experiment aimed at characterizing the effects elevated mating rate has on female longevity and lifetime offspring production. By controlling for male harassment and by using two distinct strains, we are able to assess the effect of copulation frequency per se and whether different genotypes exhibit different optimal mating rates.

METHODS

We used two allopatric wild-type strains of *C. maculatus* (Coleoptera, Bruchidae), both originating from Brazil. One strain was provided by the University of London (Peter Credland) and the other by the University of Leicester (Robert Smith). These two strains were selected from a total of six wild-type strains because preliminary experiments indicated that mating behavior, offspring production, and lifespan differed maximally between them. Both strains have been maintained in the laboratory since the mid 1970s at population sizes of 250–400 individuals (non-overlapping generations), under food and temperature conditions identical or very similar to those used in the current study (see Fricke and Arnqvist 2004). We maintained our stock cultures in large jars (1 l) on black-eyed beans (*Vigna unguiculata*). Stock cultures and experimental beetles were both kept in climate chambers at 30°C, 12-h light/12-h dark condition, and 60% relative humidity, but all matings were carried out at room temperature. To assure that only virgin females and males were used in the experiment, experimental individuals

were hatched in virgin chambers. Each of these chambers contained a single bean infested with *C. maculatus* larvae, and chambers were checked several times per day for emerging adult beetles. Adults hatched as singles and those hatched together with same sex individuals were deemed virgins and stored individually until the start of the experiment.

Rather than varying the number of copulations early in life, we experimentally varied the rate of copulation throughout the lives of females as this more closely reflects the biology of *C. maculatus*. This was achieved by exposing all experimental females to a male every day during their entire lifetime. Realized mating rate was varied by using either normal virgin males or males rendered unable to copulate at these exposures, according to the following scheme. Females were exposed to normal virgin males: (1) once only, (2) every sixth day, (3) every fourth day, (4) every third day, (5) every second day, or (6) every day. Sample sizes ranged between 27 and 34 females per treatment and strain (total $n = 354$). All females were less than 24 h of age at the start of the experiment and were mated the first time during the first day. Matings were staged in a mating vial (Petri dish, 3.5-cm diameter), and copulation usually occurred within 15 min after introducing a virgin male to a female. Successfully mated females were then randomly assigned to a treatment (treatments 1 though 6). A copulation was defined as successful when, after mounting and inserting his aedeagus into the female, the male leaned back and remained motionless for several minutes. Females that were subjected to additional mating attempts (i.e., treatments 2 though 6) were given the opportunity to mate for a maximum of 1 h during each mating opportunity. We used the following procedure: one virgin male was introduced to the female in the mating vial, and if no mating took place within 20 min, the male was replaced with another virgin male. This was repeated once more if no copulation occurred, resulting in a maximal exposure to three males at each mating opportunity. If mating did take place, the male was removed directly after the termination of the copulation, and the female was not offered another virgin male within a given mating opportunity. All matings were thus observed and recorded. To control for the potentially confounding effects of exposure to males per se, all females not scheduled to mate a given day were instead exposed for 15 min to a male whose aedeagus had been ablated. To perform the ablation, we first anesthetized males under a constant slow flow of CO_2 for 15–20 min. We then gently squeezed the males' abdomen with forceps to extrude their aedeagus, after which we removed approximately 50% of the copulatory organ by using a pair of microscissors. Ablated males harass females (i.e., chase and mount females) at rates similar to those of normal males (see Crudgington, 2001) but are unable copulate. We chose to expose females for 15 min because this corresponds to the average time until mating in trials in which mating occurred.

The mating rate treatments used here overlapped widely with the egg production period of females. The proportion of lifetime offspring production produced at different days after mating by these strains under these conditions were as follows: day 1, 36%; day 2, 20%; day 3, 12%; day 4, 10%; day 5, 7%; day 6, 5 %; and day 7 and until death, 10% (average over 20 females per strain).

To estimate female fitness, we measured the hatching rate of eggs, female lifespan, and lifetime offspring production. After the first mating, each female was transferred and kept individually in an oviposition vial (Petri dish, 9-cm diameter) containing 23 g black-eyed beans ($n \approx 90$–110) for 3 days. During the fourth day, the female was transferred to another oviposition vial provided with 18 g ($n \approx 60$–80) beans for the remainder of her life. The oviposition vials were stored

Table 1

The results of an analysis of covariance of female lifespan

Source	Sum of squares	df	F	p
Strain	9.96	1	3.41	.066
Mating treatment	21.48	5	1.47	.198
Strain × mating treatment	18.72	5	1.28	.271
Female size	53.05	1	18.19	<.001
Mating prop.	12.20	1	4.18	.042
Error	947.9	325		
Squared multiple $R = .101$				

Table 2

The results of an analysis of covariance of female lifetime offspring production

Source	Sum of squares	df	F	p
Strain	16272.3	1	53.42	<.001
Mating treatment	3688.1	5	2.42	.036
Strain × mating treatment	1779.1	5	1.17	.325
Female size	26501.6	1	87.01	<.001
Mating prop.	1494.4	1	4.91	.027
Error	99904.8	328		
Squared multiple $R = .393$				

in a climate chamber (see above). The number of adult offspring hatching from these vials were recorded. To estimate the hatching rate of eggs late in life (after day four), when differences in mating rate was most apparent across treatments, we scored 20 randomly selected eggs from the second oviposition vial of each female as either hatched or unhatched 7 days after the female's death. Hatched eggs become whitish in color, whereas eggs that fail to develop remain clear and transparent. All females were stored in 70% alcohol after their death, and their body size was subsequently measured by using a digitizing tablet placed under a side-mounted camera lucida attached to a dissecting microscope. As an integrative measure of female size, we used the first principal component in a factor analysis involving three size measures: the lengths of the two elytra and the width of the pronotum. All females were also dissected under a dissecting microscope, and the number of eggs in their reproductive tract was counted.

Statistical analyses

Females that did not produce any offspring and a few deviant females with extreme residuals (|studentized residual| > 3.5; four out of 354 females) were excluded from the analyses. Data were analyzed with conventional analyses of covariance, following the transformation of data to meet the assumptions of such models. Because not all females copulated at every exposure to normal males, we consistently used the proportion of such exposures at which mating took place for each female ("mating proportion") as a covariate in our analyses. In the models reported below, the mating treatment factor thus estimate independent effects of mating rate per se. All statistical analyses were performed with SYSTAT.

RESULTS

Our mating treatment yielded the intended effect on realized female mating rate. The average number of lifetime copulations experienced by females exposed to the different treatments were (starting with the lowest): 1.00, 1.82, 2.24, 2.59, 3.08, and 3.88 ($F_{5,347} = 70.74$, $p < .001$). Moreover, the increase in realized mating rate was linear over the six mating rate treatments (test for quadratic trend by second order polynomial contrast; $F_{1,347} = 0.01$, $p = .912$), and the realized mating rate in all mating treatments was significantly different from that in all others (Fisher's least-squares difference (LSD) post hoc contrasts, $p < .05$ in all cases).

Variance in the hatching rate of eggs laid after day 4 across females was analyzed in an ANCOVA, involving mating treatment, strain, their interaction, and two covariates (mating proportion and lifespan). Hatching rate was, however, not significantly affected by any of these factors (all effects $p > .05$). The fact that mating treatment ($F_{5,331} = 0.843$, $p = .520$), mating proportion ($F_{1,331} = 0.055$, $p = .815$),

or lifespan ($F_{1,331} = 0.027$, $p = .869$) was not related to hatching rate suggests that females did not suffer from a depletion of viable sperm in our experiment (see Arnqvist and Nilsson, 2000). Average egg hatching rate was 0.91 (SE = 0.01).

Female lifespan

Female lifespan was not significantly affected by our mating treatment (Table 1). Female size was positively, and the mating proportion weakly negatively, related to lifespan. It is, however, worth noting that variation in lifespan across females was to a large extent unrelated to any of the factors manipulated or measured in our experiments: the model presented in Table 1 accounted for only about 10% of the variance in female lifespan.
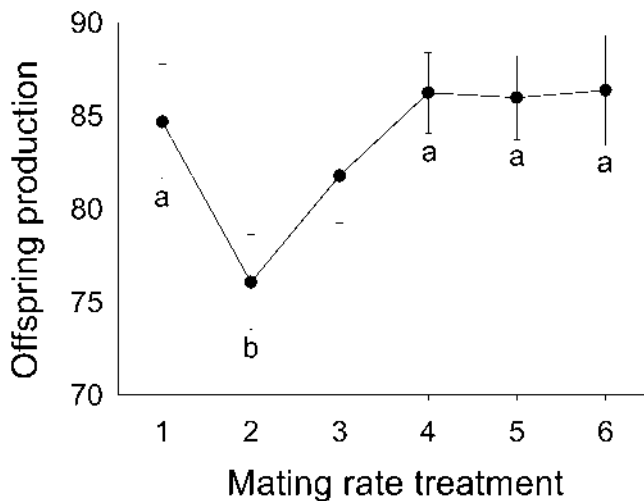
Female offspring production

The mating rate treatment had a significant effect on our measure of female net fitness (i.e., lifetime offspring production), as did female size (Table 2). Interestingly enough, our analyses showed that female fitness was the lowest at an intermediate mating rate (i.e., treatment 2), whereas females that mated either once only or more often fared equally well (Figure 2). We also note that the significant difference between treatment 1 and 2 must involve effects late in life, when the difference in mating rate between these two treatments were realized.

As might be expected for a capital breeder such as bean weevils (Tatar et al., 1993), which do not need to feed during their adult reproductive life, offspring production early in life was negatively related to offspring production late in life across all females ($n = 347$, $r = -.333$, $p < .001$; partial correlation after controlling for body size $r_{YY·X} = -0.466$). Females mated at low rates also tended to have more eggs retained in their abdomen after death (Spearman rank correlation between treatment and retained eggs; $r_s = -.121$, $p < .05$), suggesting that these females more frequently died before laying all mature eggs.

It is worth noting that although females from the London strain produced more offspring compared with those of the Leicester strain (90.8 ± 1.42 versus 76.3 ± 1.34), strain did not interact with our mating rate treatment (Table 2). Thus, the relationship between female mating rate and fitness did not vary significantly across strains (cf. Crudgington, 2001; Eady and Brown, 2000).

DISCUSSION

In the present study, we have demonstrated that female lifetime offspring production may be maximized at either of

Figure 2
Average lifetime offspring production (least-squares mean ± SE) of females exposed to increasing mating rates. Females mating once or many times fared equally well, whereas those mating at an intermediate rate produced fewer offspring (letters indicate differences at a =.05; Fisher's LSD post-hoc contrasts). For sample sizes and a description of the mating rate treatments, see Methods.

two alternative mating rates in two strains of *C. maculatus*. Those maxima occurred at a low mating frequency (a single copulation) and at high mating frequencies (three or more copulations), whereas an intermediate mating rate resulted in depressed offspring production. We did, however, find no effect of mating rate on either female lifespan or egg hatchability.

The effects of multiple mating on female fitness are clearly multifaceted in *C. maculatus* (see Introduction). Eady et al. (2000) showed that females copulating with multiple males lay more eggs, but have offspring with a lower egg-adult survival, than do those copulating repeatedly with the same male, at least under some conditions. The identity of the mate may thus affect female reproductive performance. Although our experimental design does not allow us to unambiguously separate the effects of number of matings from the potential effects of number of mates, our results do strongly suggest that mating rate per se is key to females and imply that additional direct benefits to mating (beyond sperm replenishment) occur. We can think of two such benefits, which are not mutually exclusive, both of which are consistent with the fact that male *C. maculatus* transfer a rather large ejaculate to females at mating (Savalli and Fox 1998). First, although bean weevils are capable of ingestion, they typically inhabit very arid environments and are reared under aphagous conditions in which additional water is not supplied (Labeyrie, 1981). Nevertheless, supplying water is known to prolong lifespan in the related seed beetle, *Acanthoscelides obtectus* (Leroi, 1981). This suggests that female bruchids may suffer from dehydration. The water contained in ejaculates could thus provide a benefit per se to females. Such a hydration benefit from consuming ejaculates was demonstrated by Ivy et al. (1999) in the cricket, *Gryllus sigillatus*. Interestingly enough, cricket females appeared to allocate water contained in the ejaculates toward reproduction as opposed to survival.

Second, female *C. maculatus* may benefit from metabolizing substances contained in the ejaculate (see Vahed 1998). A study by Fox (1993b) showed that multiple mating increased female lifespan when reared without food but that this effect

disappeared when females had unlimited access to water and nutrients. This suggests that multiple mating by females is advantageous under aphagous conditions (see also Takakura 1999). We note, however, that we failed to document an effect of increased mating rate on female lifespan. It has been shown in closely related Bruchid beetles that substances in the male ejaculate are incorporated into both female somatic and reproductive tissues (Boucher and Huignard, 1987; Huignard, 1983). Although Bruchid beetles feed little if anything under natural conditions and do well under aphagous conditions in the laboratory, females of several species are known to live longer and/or lay more eggs when provided with water, sugars, and/or proteins (Leroi, 1981; Tatar and Carey, 1995). Interestingly enough, the experiments of Leroi (1981) showed that a temporally limited access to water/food is insufficient to achieve such beneficial effects. This is consistent with a nonlinear relationship between dose, or rate of supply, and the beneficial effects of ejaculate substances. To separate hydration and nutritional effects, future experiments should assess the effects of multiple mating under different environmental conditions (e.g., humidity, water, and food supply).

Our results suggest that multiple mating is associated with several interacting direct costs and benefits in *C. maculatus* and that this could potentially result in two distinct and viable mating strategies (see also Wedell et al. 2002). Females could be efficient "cost minimizers" by mating at very low rates. Although providing sufficient amounts of sperm, accepting only one mating would minimize the direct cost of copulation resulting, for example, from injury to their genital tract by aedeagal spines in males (Crudgington and Siva-Jothy, 2000). Because such females would not have to allocate much resources to wound repair, they could maintain a high egg production rate early in life but may also be unable to realize their full reproductive potential because of a lack of water and/or nutrients later in life. This suggestion is consistent with the fact that females mated at low rates tended to have more unlaid and incompletely developed eggs retained in their body at the time of death. On the other hand, females could instead be "benefit maximizers" by mating with several males. Although multiply mating females will experience higher direct costs of mating, owing to, for example, elevated injury to their genital tract, they will also receive larger amounts of water and/or nutritional substances from the ejaculate. Depending on the relative rate at which such direct costs and benefits accumulate, two discrete fitness peaks may occur (Figure 1). At intermediate mating rates, the additional benefits do not seem high enough to counterbalance the costs of mating.

Earlier studies have found either positive (Fox, 1993b) or negative (Crudgington, 2001) effects of multiple mating on female longevity. We failed to find any such effects. It is interesting to note that the meta-analysis by Arnqvist and Nilsson (2000) showed that multiple mating generally decreases female longevity in insects with no nutritional direct effects of mating, whereas female longevity is generally more or less unaffected by multiple mating in insects with such effects. It thus seems that most resources that derive from the male ejaculate are generally allocated toward increased female fecundity rather than survival. This is also consistent with the results of the current study.

To our knowledge, this is the first study to document multiple distinct fitness peaks with regard to female mating rate. Because very few studies of the effects of polyandry have used a range of different female mating rates, it is difficult to assess how general such relationships may be. If several interacting direct effects commonly occur, nontrivial relationships between female mating rate and fitness may be quite

wide spread. This could certainly contribute to the maintenance of intraspecific variation in the degree of polyandry seen in some insects (Wedell et al., 2002) but would also help explain the often impressive interspecific variation in polyandry seen within higher taxa (e.g., genera, families). It is interesting to note that such interspecific variation is extensive in bruchids, in which females of some species (e.g., *Bruchidius dorsalis*; Takakura, 1999) readily mate more than 10 times during the first week of their life whereas others (e.g., *A. obtectus*; Huignard, 1974) rarely mate more than once during their entire life.

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