

The effect of male mating history on paternal investment, fecundity and female remating in the seed beetle *Callosobruchus maculatus*

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Summary

1. In many organisms, males provide nutrients to females via ejaculates that can influence female fecundity, longevity and mating behaviour. The effect of male mating history on male ejaculate size, female fecundity, female longevity and female remating behaviour in the seed beetle *Callosobruchus maculatus* was determined.
2. The quantity of ejaculate passed to females declined dramatically with successive matings. Despite the decline, a male's ability to fertilize a female fully did not appear to decline substantially until his fourth mating.
3. When females multiply mated with males of a particular mated status, the pattern of egg production was cyclic, with egg production increasing after mating. Females multiply mated to virgins had higher fecundity than females mated to non-virgins, and females mated to twice-mated males had disproportionately increased egg production late in their life.
4. Females that mated to multiple virgins, and consequently laid more eggs, experienced greater mortality than females mated only once or mated to non-virgins, suggesting that egg production is costly, and rather than ameliorating these costs, male ejaculates may increase them by allowing or stimulating females to lay more eggs.
5. Females mating with non-virgin males remated more readily than did females mated to virgins. Females given food supplements were less likely to remate than females that were nutritionally stressed, suggesting that females remate in part to obtain additional nutrients.

Key-words: Bruchidae, ejaculate size, longevity, multiple mating, nutrients

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Introduction

In insects and many other organisms, males can provide considerable nutrients to females via ejaculates, spermatophores or nuptial gifts (Thornhill 1976; Thornhill & Alcock 1983; Gwynne 1997). These nutritive contributions can impact an animal's life-history and reproductive strategies (Trivers 1972; Dewsbury 1982) by influencing female fecundity, offspring size or quality, female mating behaviour and female longevity (Parker & Simmons 1989; Boggs 1990; Wedell 1996). For example, females obtaining more or larger spermatophores may lay more or larger eggs (e.g. Markow & Ankney 1984; Gwynne 1988; Ridley 1988; Andersson 1994; Fox, McLennan & Mousseau 1995a; Eberhard 1996), resulting in fecundity selection favouring males that can produce large ejaculates or spermatophores (e.g. Savalli & Fox 1998a). When male-provided nutrients are used by

females for somatic maintenance or egg production, females should exhibit behaviours that increase the amount of nutrients that they obtain, such as choosing males that can provide more nutrients or remating with additional males (Trivers 1972; Andersson 1994).

Male nutritive contributions can vary considerably within and among individuals. They can vary with male size, with large males typically providing larger packets (e.g. Steele 1986; Wicklund *et al.* 1993; Savalli & Fox 1998a), but can also vary independently of size owing to genetic variation among males (Savalli & Fox 1998b) or to variation in the environmental conditions experienced by males (Wedell 1996). An individual male's contribution can also vary over time as he ages (Fox *et al.* 1995a), in response to social conditions (Gage & Baker 1991; Gage & Barnard 1996; He & Miyata 1997), or as a function of his mating history, with virgins generally providing more material than previously mated males

(Boggs 1981; Rutowski, Gilchrist & Terkanian 1987; Fox *et al.* 1995a). Nutritive contributions can also vary in quality or composition rather than just size (Marshall & McNeil 1989). Females can thus increase nutrient gain by selectively mating with large or high-quality males, by mating with virgins, or by mating more often (Fox 1993a; Fox & Hickman 1994).

Much of the research on the importance to females of nutrients in ejaculates or spermatophores has compared females mated with single vs multiple virgin males (see Fox 1993a; Wedell 1996; LaMunyon 1997 for recent examples). Although many studies have demonstrated a decline in male contributions with subsequent matings, few have investigated the consequences of those reduced contributions to female fecundity or the behavioural response of females to such reduced contributions. Since multiple mating by males is common (Dewsbury 1982; Thornhill & Alcock 1983; Andersson 1994), using only virgins may not accurately reflect typical situations in nature. In this study, we examine the effect of the reduced ejaculate size of non-virgin males on female fecundity, longevity and mating behaviour in a seed beetle, *Callosobruchus maculatus*.

In seed beetles (Coleoptera: Bruchidae), males produce large ejaculates and nutrients from these ejaculates are incorporated into both somatic and reproductive tissues of females (Huignard 1983; Boucher & Huignard 1987). These nutrients appear to influence larval development – females mated to multiple virgin males have greater lifetime fecundity (Wasserman & Asami 1985; Fox 1993a; but see Credland & Wright 1989), lay larger eggs (Fox 1993b) and live longer than females mated only once (Fox 1993a). Ejaculate size – and the number of sperm it contains – varies among males (Eady 1995; Savalli & Fox 1998a,b) and, within individuals, varies with age (Fox *et al.* 1995b) and male mated status: both ejaculate size and number of sperm inseminated decrease substantially with subsequent matings (Eady 1995; Fox *et al.* 1995b).

The goals of this study were: (1) to determine the effect of male mating history on male ejaculate size in the seed beetle *C. maculatus*; (2) to determine if females mating with virgin males lay more and larger eggs than females mating with previously mated males; and (3) to determine if females compensate for the reduced ejaculate size of non-virgins by remating more readily.

Population origin, maintenance and general methods

Callosobruchus maculatus is a cosmopolitan pest of stored legumes (Fabaceae). Females cement their eggs to the surface of host seeds (Messina 1991) and larvae burrow into the seed under the egg. Larval development and pupation are completed entirely within a single seed. Emerging adults are well adapted to storage conditions, requiring neither food nor water to reproduce. All beetles used in these experiments were collected from

infested pods of Cowpea (*Vigna unguiculata*) in Niamey, Niger, at the University of Niamey Experiment Station, in November 1989, and maintained in laboratory growth chambers at > 1500 adults per generation prior to this experiment. During the experiments, beetles were maintained at 26 °C with 24 h light.

All experiments were initiated with virgin males and females collected from isolated Cowpea seeds within 12 h of their adult emergence. Because males emerge with only partially filled seminal vesicles (Fox *et al.* 1995b), they were allowed to mature for 24 h before being used in any experiments. Thus, all virgin beetles were isolated from each other in individual 35-mm Petri dishes without seeds for 24 h before being used, such that all parents were of similar age, between 24- and 36-h-old. Each beetle was used only once. Immediately prior to each experiment, all beetles were weighed on an electronic balance to 0.1-mg precision (fecundity and mating behaviour experiments) or 0.01-mg precision (ejaculate size experiment).

The effects of mating with virgin or non-virgin males

EXPERIMENT 1: THE EFFECT OF MALE REMATING ON EJACULATE SIZE

Methods

To determine whether a male's ejaculate size changes with successive matings, virgin males were mated consecutively to three females and mass loss during mating was measured. To control for male body size (which affects ejaculate size; Savalli & Fox 1998b), a paired design was used in which 11 males were each mated sequentially to 3 females within a 1-h period. The first female in this trio thus mated with the male when he was a virgin, the second, after he had previously mated once (hereafter referred to as a 'once-mated male'), and the third, after he had mated twice before (a 'twice-mated male'). To determine ejaculate size, beetles were weighed twice to 0.01-mg precision on an electronic balance both before and after each mating. If the two values differed by > 0.04 mg, a third weighing was performed. A beetle's mass was estimated as the average of these two or three values. Ejaculate size was estimated as the mass lost by the male during mating. Metabolic mass loss is unlikely to confound these measurements since all experiments were carried out in similar laboratory conditions and metabolic mass loss is undetectable for the duration of typical matings (U. M. Savalli & C. W. Fox, unpublished data).

Results

Virgin males contributed more ejaculate to females than did once- or twice-mated males (repeated-measures ANOVA, $F_{2,20} = 91.5$, $P < 0.001$; Fig. 1). Male contributions constituted a substantial proportion of their pre-mating body mass (first mating, mean \pm SE = $5.3 \pm 0.3\%$;

second mating, $3.2 \pm 0.3\%$; third mating, $1.4 \pm 0.1\%$). If male *C. maculatus* do contribute nutrients in their ejaculate, as has been suggested by several authors (Wasserman & Asami 1985; Credland & Wright 1989; Fox 1993a), then females mating with non-virgins may obtain fewer nutrients during mating than females mating with virgins, and may suffer reduced fecundity or survivorship as a result.

EXPERIMENT 2: THE EFFECT ON FECUNDITY OF MATING ONCE WITH A VIRGIN OR NON-VIRGIN MALE

Methods

In the first experiment it was established that males mated previously transfer substantially smaller ejaculates than unmated males. To test if such reduced ejaculates affect female fecundity and fertility, 22 initially virgin males were, in quick succession, sequentially mated to 4 females, each matched for size to within 0.6 mg. Each female was mated only once. Each female was then placed alone in a 60-mm Petri dish with ≈ 70 Cowpea seeds and allowed to lay eggs. After 48 h, females were transferred to a new dish with ≈ 70 fresh seeds and allowed to lay eggs until they died. All the eggs were allowed to develop for at least 7 days before scoring for fertile and infertile eggs under a dissecting microscope. Infertile eggs

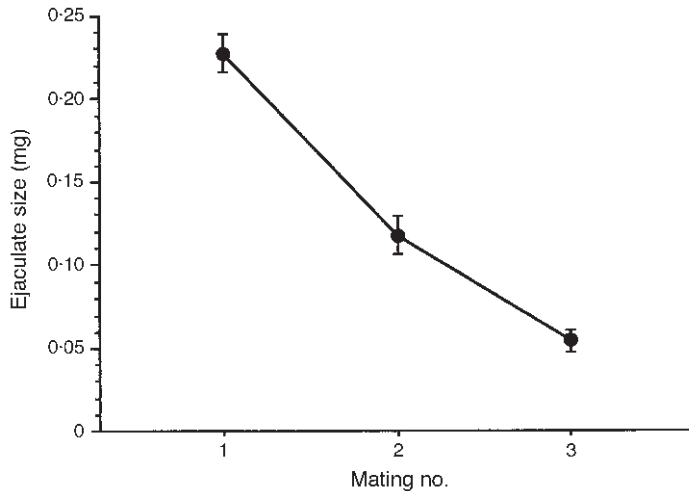


Fig. 1. The effect of repeated mating on male ejaculate size (percentages given in text). Error bars represent 1 SE.

Table 1. The effect of mating order on the ability of males to fertilize females (females were considered fertile if they laid one or more eggs that developed into larvae) and on female lifetime fecundity (fertile females only). See text for statistics

| | Fertilization order | | | |
|--------------------------------|---------------------|-------------------|-------------------|-------------------|
| | First | Second | Third | Fourth |
| No. of fertile females | 21 | 19 | 21 | 13 |
| No. of infertile females | 1 | 2 | 0 | 6 |
| Lifetime fecundity (\pm SE) | 89.4 (\pm 3.4) | 75.3 (\pm 3.5) | 76.8 (\pm 4.9) | 45.4 (\pm 9.1) |

(and fertile eggs that fail to develop) remain clear, while developing eggs either contain a visible larva (noticeable by the black, sclerotized head), or, once the larvae hatch and burrow into the seed, are filled with white frass. Because some males failed to mate all four times, a repeated-measures ANOVA could not be used. Instead, an individual male block was included in an analysis of variance (Sokal & Rohlf 1981).

Results

Some females did not lay any fertile eggs and thus probably did not receive any sperm during mating: the males' first to third matings all resulted in most of the females being fertilized, while the fourth mating resulted in significantly fewer females being fertilized (Table 1; $\chi^2 = 11.95$; $df = 3$; $P = 0.008$). Thus, there was little consequence of mating with a once- or twice-mated male on whether or not females were fertilized, but mating to a male that had mated more than three times previously reduced a female's chance of being fertilized.

Of those females that were fertilized, females mated to virgin males had slightly, but non-significantly ($0.1 > P > 0.05$), higher fecundity than those mated to previously once- or twice-mated males, and females from a male's fourth fertilization had significantly lower fecundity ($P < 0.05$, Newman-Keuls *post-hoc* comparisons; overall, $F_{3,50} = 11.52$; $P < 0.001$; Table 1), probably due to, at least in part, insufficient sperm being transferred.

EXPERIMENT 3: THE EFFECT ON FECUNDITY AND EGG SIZE OF MATING MULTIPLE WITH VIRGIN OR NON-VIRGIN MALES

Methods

Experiment 2 suggested that females may gain a slight advantage from mating with a virgin male in the form of increased frequency of successful fertilization and increased fecundity. However, females were only allowed to mate once; in the laboratory females remate readily after a refractory period and thus probably remate in nature. In this experiment the potential effect of mating with previously mated males was enhanced by allowing females to multiply mate with males of a particular mated status. Since males paired four times were often unable to fertilize the fourth female and did not always mate with the fourth female, males were limited to three sequential matings.

As in expt 2, 50 initially virgin males were sequentially mated to 3 females, with each trio of females matched for size to within 0.3 mg. The females were then placed in a 60-mm Petri dish with ≈ 35 Cowpea seeds and allowed to lay eggs. Because egg size declines with female age (Fox 1993b; Fox & Dingle 1994), females were transferred to a new dish with seeds after 12 h and allowed to lay eggs for another 36 h. The

females were then mated, in the same sequence, to a new male, so that females initially mated to a virgin male were remated to a virgin, females mated to a once-mated male were remated to another once-mated male, and so on. If a female did not mate (4% of all re-pairings, distributed among all three treatments), the male was mated to a 'stand-in' female that was subsequently discarded so that he would have the appropriate mated status for the next female. The females were once again placed on fresh seeds that were then changed after 24 h. Females were mated every 48 h in this manner a total of four times, with the seeds being replaced every 24 h. After the last mating, females were left on the same seeds until death and checked for mortality every 12 h.

For comparison, a fourth female, matched in size to the other three, was mated at the same time as the others to a virgin male that was also matched in size to the first; these females were given fresh seeds on the same schedule as the other females but were not remated. All the eggs were allowed to develop until after hatching and hatched eggs (containing frass) in each dish were then counted. For each female the length and width of two randomly chosen eggs laid in the first interval (0–12 h) and two laid in the sixth interval (120–144 h) were measured using an ocular micrometer on a stereomicroscope.

As in expt 2, a 'replicate' block was included in all analyses, in which each replicate was the set of four matched females and the males to which they were sequentially mated.

Results

Lifetime fecundity was greatest for females that mated repeatedly with virgin males, and decreased as

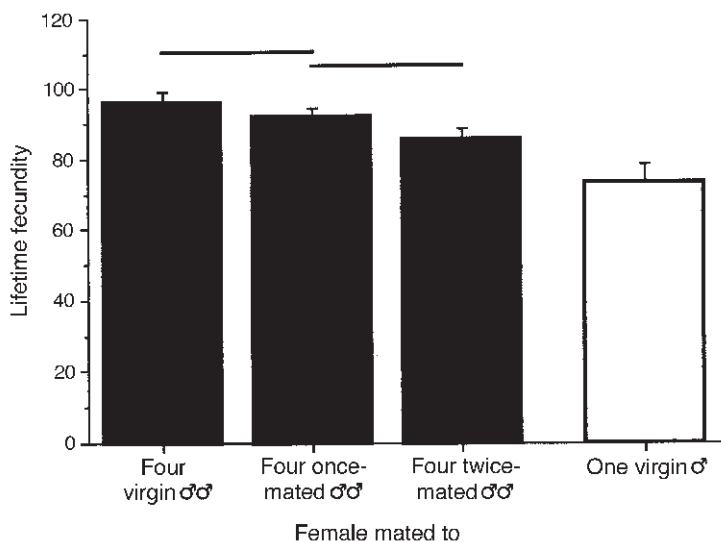


Fig. 2. The effect of female mating treatment (mated once vs mated four times; mated to virgin, once-mated or twice-mated males) on the total number of eggs laid in a female's lifetime. The horizontal bars indicates means that are not statistically significantly different (Newman–Keuls *post-hoc* tests; $P > 0.05$). $N = 47$ – 50 females per treatment. See text for statistics. Error bars represent 1 SE.

the males' number of previous matings increased (excluding the females that mated only once, $F_{2,92} = 5.57$, $P = 0.005$; Fig. 2). Lifetime fecundity was lowest for females mated only once (all four treatments, $F_{3,142} = 11.62$, $P < 0.001$; Fig. 2). Thus, females mated once to a virgin male did more poorly than females mated to four twice-mated males, despite the fact that both of these females appear to get similar amounts of ejaculate (0.23 mg for females mated to a virgin vs 4×0.054 mg = 0.22 mg for females mated to four non-virgins; calculated using data from expt 1). There were no differences in egg size between the treatments (first interval, egg length, $F_{3,123} = 0.11$, $P = 0.96$; egg width, $F_{3,123} = 0.20$, $P = 0.90$; sixth interval, egg length, $F_{3,91} = 0.61$, $P = 0.61$; egg width, $F_{3,91} = 2.20$, $P = 0.093$).

Examining the fecundity data for each 24-h interval reveals a complex pattern of egg-laying (Fig. 3). For all three multiple-mating treatments, egg-laying rates are higher in the first 24-h period following each mating than in the second 24-h period after mating (Fig. 3; note that the number of eggs for the first two intervals – which are 12 and 36 h, respectively – have been corrected to eggs/24 h, and that the last interval lasted for the rest of the female's life). Thus, females show a drop in egg-laying rates 12–24 h after mating and this pattern was observed for all four matings, well before females reach their total lifetime egg production. This pattern is not due to normal periodicity in egg-laying rates since it did not occur in once-mated females.

There were also clear treatment differences in the rates of egg production (Table 2; Fig. 3): although females initially mated to twice-mated males had lower egg production rates during the first 48 h than did females mated to virgins or once-mated males ($P < 0.001$ for both intervals), immediately following the second mating they did not differ ($P > 0.05$; Fig. 3). In the second 24-h interval following the second mating, egg-laying rates for females mated to twice-mated males was again lower than for females mated to once-mated or virgin males ($P < 0.05$), but in all subsequent intervals there either was no difference (following the third mating, $P > 0.05$ for both intervals) or egg production was actually higher for females mated to twice-mated males (last interval; $P < 0.05$). Thus, although egg production rates after the first mating were substantially lower for females mated to twice-mated males, these females could partially compensate for small initial ejaculates with greater egg production rates following subsequent matings.

Females mated to virgins had shorter lifespans than those mated to once- or twice-mated males (excluding the females that were mated only once, $F_{2,92} = 5.16$, $P = 0.008$; Fig. 4). Lifespan was longest for females mated only once (all four treatments, $F_{3,142} = 7.74$, $P < 0.001$; Fig. 4). These results are opposite those reported by Fox (1993a) for a different population of

Table 2. Analysis of variance of the effect of time interval (see text and Fig. 3) and mating treatment (females mated four times each to virgins, once-mated, or twice-mated males) on the number of eggs laid per interval. 'Replicate' represents each individual trio of females and the four males to which they were sequentially mated

| | df | F | P-value |
|------------------------------|-----|-------|---------|
| Time interval | 6 | 18.98 | <0.001 |
| Mating treatment | 2 | 4.63 | 0.010 |
| Replicate | 49 | 0.68 | 0.954 |
| Treatment × time interaction | 12 | 4.42 | <0.001 |
| Residual | 957 | | |

C. maculatus, in which multiply mated females not only had greater fecundity (as in this study) but also lived longer than singly mated females.

Female remating behaviour

EXPERIMENT 4: EFFECT OF MALE MATING HISTORY ON FEMALE REMATING

Methods

One way that females can compensate for the reduced ejaculate size or reduced number of sperm obtained by mating with non-virgin males is to remate sooner with other males. To test whether females attempt to

compensate for male mating history, the tendencies of a female to remate when she encountered a new male if she had first mated with a virgin male (with full seminal vesicles) or with a non-virgin male (with partially depleted seminal vesicles) were compared. Males were sequentially mated to three randomly selected virgin females. To stimulate females to remate, females were placed on Cowpea seeds and allowed to lay eggs. Thus, once mated, each female was placed alone in a 35-mm Petri dish with ≈ 10 seeds for 16 h, after which each of these females (mated to a virgin, once-mated and twice-mated male, respectively) was confined with a new, randomly selected virgin male and monitored for 15 min. Whether a female exhibited rejection behaviours (consistently running away from a male that has contacted her, or kicking at a male that is attempting to mount) and whether or not she remated were recorded. In subsequent statistical analyses it was tested whether females that mated first with a virgin male were less likely to remate than females that first mated with the same male when he was non-virgin.

Results

The mated status of the first male to mate with a female affected her tendency to remate: females mated to twice-mated males almost always remated

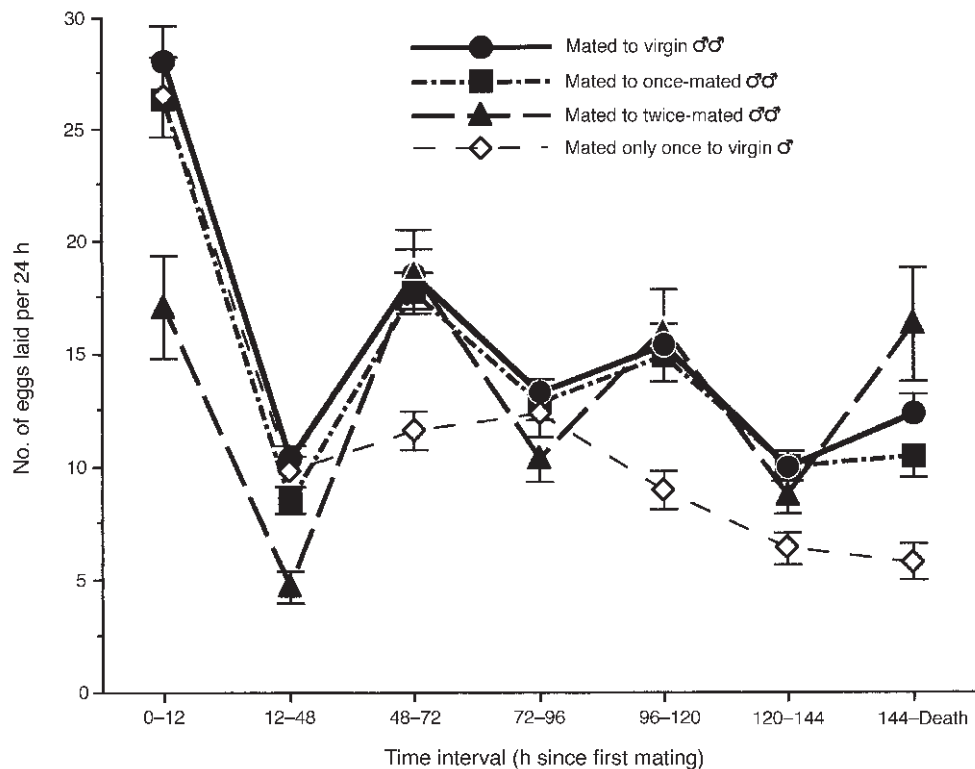


Fig. 3. The effect of female mating treatment (solid points: mated four times to virgin, once-mated or twice-mated males; open diamonds: mated once to a virgin male) on the number of eggs laid in each successive time interval. Note that the first two intervals have been corrected to give the number of eggs/24 h rather than the number of eggs/dish, but the last interval represents the total number of eggs laid per dish until the female's death. $N = 47-50$ females per treatment. See Table 2 and text for statistics. Some standard error bars have been omitted to reduce clutter.

after 16 h, while females initially mated to a virgin male more often than not did not remate ($\chi^2 = 23.1$, $df = 2$, $P < 0.001$; Table 3A). In 70% of the remating trials in which females did not remate, the female had actively rejected the male that attempted to mate. Females initially mated to virgin males rejected the second males far more often than did females mated to non-virgin males ($\chi^2 = 26.9$, $df = 2$, $P < 0.001$; Table 3B; rejections include four cases where females initially rejected the male but then mated with him). These results demonstrate that females mated to non-virgin males remate more readily with subsequent males.

EXPERIMENT 5: EFFECT OF FOOD SUPPLEMENTS ON FEMALE REMATING

Methods

The previous experiment demonstrated that females mated to previously mated males, and thus receiving

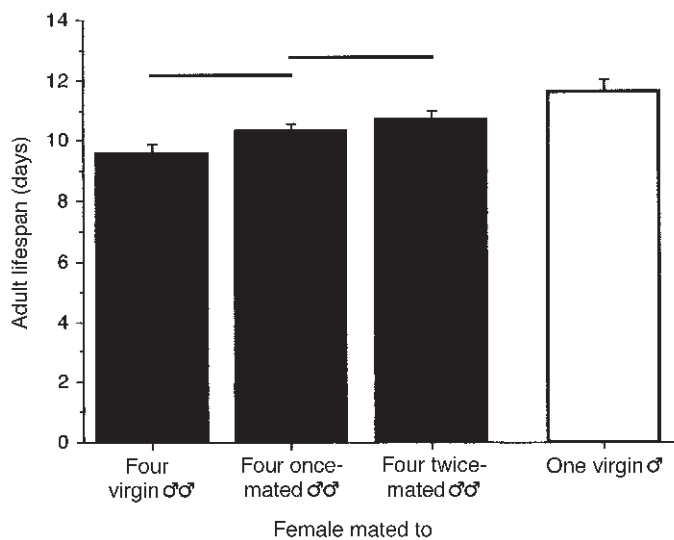


Fig. 4. The effect of female mating treatment (solid bars: mated four times to virgin, once-mated or twice-mated males; open bar: mated once to a virgin male) on female adult lifespan. The horizontal bars indicates means that are not statistically significantly different (Newman-Keuls *post-hoc* tests; $P > 0.05$). Sample sizes as in Fig. 3. See text for statistics. Error bars represent 1 SE.

Table 3. The effect of the mated status of the first male to mate with a female on (A) whether or not she remated with a second male; and (B) whether or not she exhibited rejection behaviours (running, kicking) towards the second male

| | First male mated status | | |
|------------------------|-------------------------|------------|-------------|
| | Virgin | Mated once | Mated twice |
| A | | | |
| Females remated | 10 | 20 | 26 |
| Females did not remate | 21 | 11 | 2 |
| B | | | |
| Females did not reject | 11 | 27 | 25 |
| Females rejected male | 20 | 4 | 3 |

smaller ejaculates, remated more readily than females mated to virgins. This difference could be because (1) male ejaculate contains a chemical that inhibits female remating, with large ejaculates containing more of that chemical (Chen 1984; Eberhard 1996); (2) females remate to obtain additional sperm; or (3) females remate to obtain more nutrients. To test the latter hypothesis, the effect of providing supplemental food on the tendency of females to remate was examined.

Fifty males were each sequentially mated to three randomly selected virgin females. As in expt 4, females were placed on seeds after mating and allowed to lay eggs for 16 h. The females were then removed from the seeds and half of the females from each mating treatment were randomly assigned to either the food supplement or food-deprived treatment. The food-supplemented females were each given dry baker's yeast and a vial of 5% sucrose solution (provided in 2.75-ml shell vials stoppered with cotton wool) for 12 h, while the food-deprived females were kept in an empty Petri dish for the same time period. All the females were then tested by pairing them with new virgin males for 15 min and recording whether or not they mated, as in expt 4.

Results

As in the previous experiment, females mated to virgin males were less likely to remate than females mated to previously mated males, pooling across both food treatments ($\chi^2 = 8.09$, $df = 2$, $P = 0.018$; Table 4). Note that within just the food-deprived treatment there was a trend towards females mated to virgin males remating less often than females mated to previously mated males (as observed in expt 4), but that the magnitude of the effect and sample size was smaller than in expt 4, and no statistically significant effect ($\chi^2 = 1.48$, $P = 0.48$) was detectable. This difference may be due to the fact that there was a longer duration between matings in this experiment (because of the need to feed the beetles) compared with expt 4, making females more likely to mate regardless of treatment.

Females that were mated to virgin males and that received a food supplement were less likely to remate than females that did not receive a food supplement ($\chi^2 = 6.88$, $df = 1$, $P = 0.009$). There was no effect of food treatment on females mated to non-virgin males (once-mated males, $\chi^2 = 0.80$, $df = 1$, $P = 0.37$; twice-mated males, $\chi^2 = 0.33$, $df = 1$, $P = 0.57$). These results suggest that females remate at least in part to obtain additional nutrients in times of nutritional stress, although when severely stressed, such as when mated to an already-mated male, additional effects of food supplementation were not detectable.

Discussion

The importance of male nutritive contributions to female reproduction has been examined in a variety of

insects (e.g. Thornhill & Alcock 1983; Markow & Ankney 1984; Boggs 1990; Andersson 1994; Eberhard 1996; Gwynne 1997), but most studies have used only virgins, thereby providing an incomplete picture of male and female reproductive tactics. In this study it has been demonstrated that, in *C. maculatus*, male mated status can have substantial effects on the size of the ejaculate that a male is able to contribute – dropping more than 75% between the first and third matings – which in turn affects female fecundity and longevity. Furthermore, it has been shown that females are not passive receptacles for male sperm and nutrients, but alter their mating behaviour in response to male mated status, remating more readily if they receive a smaller ejaculate.

The dramatic decline in quantity of ejaculate passed to females with subsequent matings is similar to the successive decline in the number of sperm transferred (quantified for *C. maculatus* by Eady 1995), suggesting that at least some of the decline in mass results from fewer sperm being transferred. Despite the decline in ejaculate volume between the first and third matings, however, a male's ability to fertilize a female fully did not appear to decline until his fourth mating. This is probably because males transfer far more sperm than females can use: on their first ejaculate, males inseminated seven times as many sperm ($\approx 46\,000$) as could be retained in the females' spermathecae (≈ 6500); the remainder rapidly degraded (Eady 1994). Indeed, even on their fourth mating, male *C. maculatus* inseminated more sperm (≈ 8700) than could be stored in the spermatheca (Eady 1995). Eady (1995) also did not detect any effect of first male mating history on female fertility or on sperm precedence (the second male to mate obtains ≈ 80 – 90% of all fertilizations), suggesting that, in his population, even by the fourth mating males inseminate sufficient sperm to fertilize all of his mate's eggs. The mating history of the second male did affect sperm precedence however, suggesting that the excess sperm may function in sperm competition (Eady 1995). Our population may differ from Eady's in that, by their fourth mating, at least some males appear to be depleted of sperm since 32% of these matings did not result in any fertilized eggs and the fecundity of the females that did receive sperm was lower than for females mated to less-often mated males.

When females were allowed to mate multiply with males of a particular mated status (expt 3), the results were similar: females mated to virgin males had higher fecundity than females mated to non-virgin males. Interestingly, females mated to twice-mated males had disproportionately high egg production late in their life, compared with females mated to once-mated or virgin males. These results are consistent with five hypotheses. First, females may exhibit cryptic choice, using ejaculate size to evaluate males (ejaculate size of virgin males is correlated with body size; Fox *et al.* 1995b; Savalli & Fox 1998b); those receiving smaller ejaculates may hold back egg production to increase the chance of having their eggs inseminated by a subsequent male with a larger ejaculate. This hypothesis is consistent with the observation that females obtaining smaller ejaculates remate more readily. Once these females reach the end of their fertile period they may need to settle for using the sperm already obtained, resulting in the release of eggs that had been retained. Second, females mated to non-virgin males may not obtain sufficient sperm to fertilize all their eggs (e.g. Royer & McNeil 1993). The high rate of egg production late in the life of these females may result from an accumulation of eggs while they wait for additional sperm, compared with the females mated to virgin males, which have enough sperm to fertilize eggs at rates closer to their rate of egg maturation. However, the results from expt 2, and the observation that males inseminate more sperm than females need to fertilize eggs (Eady 1994, 1995), suggest that sperm are not limiting until a male's fourth mating. Third, females mated to virgin males may obtain more nutrients that can be used to increase the rate of egg maturation early in life relative to females mated to non-virgins, thereby maturing a greater proportion of their eggs early on. Females mated to non-virgins do not receive as many nutrients and must mature their eggs more gradually, resulting in proportionately more eggs laid later in life. Fourth, male ejaculates may contain a substance that stimulates female egg production in a dosage-dependent manner (Chen 1984; Spencer *et al.* 1995; Eberhard 1996) so that females that obtain larger ejaculates will be more stimulated to increase the rate of egg maturation and thus mature more eggs early in life. Females receiving small ejaculates would be less stimulated and would mature eggs more gradually, leaving more eggs available later in life. Lastly, it is possible that males may be able to detect a female's remaining egg supply or her age and adjust their ejaculates accordingly. Thus, when mating with older, already-mated females, a virgin male may contribute smaller ejaculates, leaving more ejaculate (containing more sperm and/or more nutrients) for his subsequent matings.

The periodicity of egg production seen in expt 3 in which females increase egg-laying immediately following mating is consistent with the latter three hypotheses: females may use nutrients in the male's

Table 4. The effect of female nutritional status and the mated status of the first male to mate with a female on whether or not she remated with a second male

| | | First male mated status | | |
|--------------------------|----------------|-------------------------|------------|-------------|
| | | Virgin | Mated once | Mated twice |
| Received food supplement | Remated | 5 | 15 | 13 |
| | Did not remate | 20 | 10 | 12 |
| Were food deprived | Remated | 14 | 18 | 15 |
| | Did not remate | 11 | 7 | 10 |

ejaculate to increase their rate of egg production temporarily, with virgin males providing more nutrients than non-virgin males; or females may be responding to some rapidly degrading chemical stimulant in the male's ejaculate, with the larger ejaculates delivered by virgin males containing more stimulant.

Females that mated to multiple virgins, and consequently laid more eggs, also had a shorter lifespan than females mated only once or mated to non-virgins. This result is consistent with many studies that have demonstrated that increased mortality is a cost of increased reproduction (Partridge & Harvey 1985; Stearns 1992; Tater, Carey & Vaupel 1993). However, it contradicts the findings of several studies (Pivnick & McNeil 1987; Rutowski *et al.* 1987; Burpee & Sakaluk 1993; Wicklund *et al.* 1993; Tamhankar 1995) – including Fox's (1993a) with another population of this species – that multiple mating by females increases longevity compared with females mated only once. It is probable that the difference between this study and Fox's (1993a) reflects population differences (Fox's population was collected from Azuki Beans [*V. angularis*] in San Francisco, CA, while our present population was collected from Cowpeas in Niger; unfortunately, the San Francisco population is no longer maintained in the laboratory) in either the nutritional content of the ejaculate or how females make use of the ejaculate. Virgin males of both populations contribute about 5% of their body mass, and the males in Fox's population were smaller than those in this study (3.6 vs 4.6 mg; females of both populations weigh \approx 5.6 mg; C. W. Fox & U. M. Savalli, unpublished data); upon re-examination, we found Fox's original data to be robust and highly statistically significant. Our result is consistent with egg production being costly, and rather than ameliorating these costs, male ejaculates may increase them by allowing (by providing sufficient sperm) or stimulating (via some hormone) females to lay more eggs (e.g. Chen 1984; Spencer *et al.* 1995; Eberhard 1996). Few studies have investigated population differences in male contributions to female mating and the consequences of these contributions.

Females mating with non-virgin males remated more readily than did females mated to virgins. This result could be because (1) male ejaculate contains an antiaphrodisiac that inhibits female remating, with large ejaculates containing more antiaphrodisiac (Chen 1984; Spencer *et al.* 1995); (2) females remate to obtain additional sperm; or (3) females remate to obtain more nutrients. The latter hypothesis is supported by the finding that females given food supplements were less likely to remate than females that were nutritionally stressed. However, these hypotheses are not mutually exclusive and we cannot discount the possibility that female remating is influenced by the presence of antiaphrodisiac compounds provided by the male or the need to replenish sperm (although we obtained little direct evidence that females mated

to once- or twice-mated males were sperm-limited).

In summary, a male seed beetle's mating history can influence female fecundity, longevity and mating behaviour. We found no evidence of sperm limitation for females mated to virgin, once-mated or twice-mated males, although males mated more than three times may be sperm-depleted. Instead, the variation in the fecundity of females mated to males of different mating histories is more likely owing a combination of a chemical signal from males to stimulate egg-laying and some male nutrient contributions. Intriguing differences were also found in the effects of multiple mating by males between our Niger population and a population from San Francisco (Fox 1993a), suggesting that there may be significant population differences in the role of male ejaculates in these and perhaps other insects. Such interpopulation variation has been little explored.

Using only virgin males in female remating and fecundity experiments provides an incomplete, and possibly inaccurate, picture of what happens in nature. Although rates of remating are unknown for wild populations of *C. maculatus*, this species is primarily associated with human legume seed stores (Messina 1991) where opportunities for remating are likely to be plentiful. Thus, females are likely to have numerous opportunities to mate multiply and to frequently encounter previously mated males. Females may get fewer benefits per mating and remate more often than studies using only virgin males would indicate.

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