Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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**Summary**

1. Females of most insect species mate frequently. Many hypotheses have been proposed to account for the evolution of multiple mating in female insects. In this paper, I test the hypothesis that females of the bruchid beetle, *Callosobruchus maculatus*, mate frequently to replenish depleting sperm supplies. I also test the hypothesis that females obtain a nutritional contribution from males during copulation, and that this has a positive effect on the female’s life history.

2. For *C. maculatus*, there is no difference in lifetime fecundity between females that mate one time and females that are confined with males throughout life. However, when females are mated at 48-h intervals, but are not confined with males, they lay more eggs than females which have mated only once.

3. When females were maintained under starvation conditions, multiple mating increased female longevity. However, when females had unlimited access to yeast and sugar–water, this influence disappeared. These results support the hypothesis that ejaculate-derived nutrients contribute to female somatic maintenance, but are only detectable when females are nutrient stressed.

*Key-words:* Ejaculate-derived nutrients, life span, mating frequency, survivorship


**Introduction**

Much of the social behaviour of sexual animals is organized around reproductive activities, and so an understanding of mating systems is important for an understanding of social behaviour. Most research on mating systems focuses on the costs and benefits of alternative mating tactics for males. It is generally assumed that mating frequently is more important to males than females because of anisogamy and the resulting sex differences in potential reproductive success. However, it is also observed that females of most insect species mate frequently (Ridley 1990).

Several hypotheses have been proposed to explain why females should mate multiply. These can be grouped into eight classes (excluding social insects): (1) multiple mating by females may be a non-adaptive correlated response to selection for multiple mating in males (Halliday & Arnold 1987); (2) females may remate to replenish depleting sperm supplies (Walker 1980) or to protect against matings involving no sperm transfer or the transfer of inviable sperm (Ridley 1988); (3) females may remate to replace sperm which may be senescing, dying (e.g. Tsubaki & Yamagishi 1991), or accumulating mutations during storage (Purdom, Dyer & Papworth 1968); (4) remating may be less costly to the female than resisting harassment by a persistent male (Parker 1970); (5) material benefits may be transferred during copulation, either via the ejaculate (Boggs & Gilbert 1979) or nuptial gifts (Thornhill 1976); (6) risk of predation or parasitism may be reduced during copulation (Chaplin 1973; Walker 1980); (7) remating may enhance competition among sperm for fertilizations (Walker 1980); or (8) remating may promote genetic variability among offspring (possibly selected for in heterogeneous habitats) (Caldwell & Rankin 1974; Williams 1975).

Females of the bruchid beetle *Callosobruchus maculatus* (Fabricius) often mate many times per day if males are present (Rup 1986; C. Fox, unpublished data). In this study, I begin to address the above hypotheses by examining the influence of multiple mating by female *C. maculatus* on rates of egg production and female survivorship. Specifically, I test the hypotheses that females obtain some nutritional benefit from males during copulation, and that females are required to mate more than once to fertilize all of their eggs.

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Materials and methods

NATURAL HISTORY OF CALLOSOBROCHUS MACULATUS

Callosobruchus maculatus is a cosmopolitan pest of stored legumes. Females colonize seeds both in the field and in storage, cementing their eggs to the surface of the host seeds. Approximately 4–5 days later (at 28°C), the eggs hatch and the first instar larvae burrow into the seed, directly beneath 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.

EXPERIMENTAL POPULATION

All beetles used in these experiments were collected from stored azuki beans (Vigna angularis) near San Francisco, California (Fox 1992). The laboratory population was established with more than 1000 eggs and maintained on azuki beans, before and during the experiment, at 27±1°C, 24h light. Beetles from the fourth laboratory generation were used in the first experiment, those from the twelfth generation were used in the second experiment, and those from the fifteenth generation were used in the third experiment.

EXPERIMENT 1: THE PRESENCE OF MALES AND FEMALE EGG PRODUCTION

Virgin females were collected from isolated beans within 24h of emergence from the seed and presented with a single virgin male in a 30-mm petri dish. Following copulation all females were measured (elytron length and pronotum width) and then transferred to 60-mm petri dishes containing 5.0g azuki seeds. Half of the females were maintained singly until death, and thus copulated only once (following emergence). The other half were confined with three males until the females died. These were observed to have copulated many times throughout the experiment (sometimes even remating within 10 min of a previous copulation).

Each 24h for the first 3 days, females (and their males) were transferred to a new petri dish with clean seeds. Dead males were replaced during these transfers. From the third day until death females were left undisturbed. No data on female life spans are available for this experiment. Lifetime egg production was recorded for all females.

All eggs (in this and each subsequent experiment) were classified as either ‘fertile’ or ‘infertile’; the latter were eggs that failed to begin development (no evidence of embryonic differentiation under a 50× dissecting scope). However, it is not known whether these eggs were actually unfertilized, or whether some were fertilized but failed to develop. In all cases, analyses of lifetime fertile egg production are qualitatively identical to analyses of lifetime total egg production, so only the latter are presented. Females laid very few eggs which failed to develop, even at older ages (the correlations between lifetime fertile egg production and lifetime total egg production are larger than 0.96 in each experiment).

EXPERIMENT 2: MATING FREQUENCY, LIFETIME FECUNDITY AND FEMALE SURVIVORSHIP

Virgin females were collected from isolated seeds and presented with virgin males within c. 12h of adult emergence. Following one mating, all females were transferred to 60-mm petri dishes containing 5.0g azuki seeds.

Instead of confining females with males (as in experiment 1), half of the females were remated periodically by transferring them into an empty 30-mm petri dish every 48h, presenting them with one virgin male less than 12h old, and allowing them to copulate once (which generally occurred within 10 min). These females were then transferred alone into a new 60-mm petri dish containing 5.0g azuki seeds. The rest of the females were transferred directly to fresh seeds daily without access to males.

Every 24h (until death) all females were transferred to a new dish containing 5.0g clean azuki seeds. Daily egg production and time of death (within 12h) was recorded for all females.

EXPERIMENT 3: MATING FREQUENCY, LIFETIME FECUNDITY AND FEMALE SURVIVORSHIP WITH FOOD SUPPLEMENTATION

This experiment was designed to test the hypothesis that the among-treatment difference in survivorship observed in experiment 2 may be due to nutrient transfer from male to female during copulation. Thus, this experiment was identical to experiment 2 except that: (1) females were provided with baker’s yeast (Red Star active dry yeast) ad libitum and a 5% sucrose solution (supplied in 2.75-ml shell vials stoppered with cotton and replaced every 48h throughout the experiment), and (2) females were transferred to new seeds daily for only the first 8 days, after which they were left in a single dish until death.

Because some females laid more eggs than anticipated from the results of experiment 2, these had to be transferred to new seeds once more (after 8 days) before their death (note that all females were transferred every 24h for the first 8 days). The exact age of females and the number of eggs they had laid prior to this last transfer varied among females. Although only females mated multiple times laid enough eggs to require additional oviposition substrate, females from both treatments were transferred to new seeds simultaneously to control for any influence of extra handling on mortality.
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![Graph showing life expectancy and egg production](image)

**Fig. 1.** Lifetime fecundity of *Callosobruchus maculatus* females in experiment 1 (females mated once or females confined with males).

Time of death (within 12 h) was recorded for all females, and daily egg production was recorded for 45 females in each treatment.

**Results**

For each experiment, results of all analyses are qualitatively identical for elytron length, pronotum width, and body weight (average correlation between pronotum width and elytron length is 0·87, between body weight and pronotum width is 0·81, and between body weight and elytron length is 0·84). Because body weight data are not available for experiment 1, only results for elytron length are presented.

**EXPERIMENT 1: THE PRESENCE OF MALES AND FEMALE EGG PRODUCTION**

The number of eggs produced by females confined with males throughout their life was not significantly different from the number of eggs produced by females that had copulated only once (Fig. 1). An analysis of covariance, with body size (elytron length) as a covariate, detected a significant influence of body size ($F_{1,103}=162·73$, $P<0·001$) on lifetime fecundity, but no significant influence of treatment (mated once or confined with males, $F_{1,103}=2·85$, $P=0·09$).

**EXPERIMENT 2: MATING FREQUENCY, LIFETIME FECUNDITY AND FEMALE SURVIVORSHIP**

When not continuously confined with males, females that mated multiply laid more eggs than females that mated only once (Fig. 2a), particularly at older ages (Fig. 2b).

One possible explanation for why females confined with males throughout their life do not lay more eggs than females mated only once is that multiple mating results in higher female mortality. However, results of the second experiment indicate that, despite laying more eggs, multiply mated females actually lived longer than once-mated females (Fig. 3a): mean life span was 8·4±0·1 days for once-mated females ($n=114$) and 9·0±0·1 days for multiply mated females ($n=115$) (Mann–Whitney $U$-test, $U=4990$, $P=0·002$). This difference among treatments cannot be explained by larger body size of multiply mated females: there were no significant among-treatment differences in body size (Mann–Whitney $U$-test, $U=6787$, $P=0·52$ for elytron length). An analysis of covariance, with body size (elytron length) as a covariate, detected both treatment (mated once or mated multiply, $F_{1,226}=11·56$, $P<0·001$) and body size ($F_{1,226}=15·56$, $P<0·001$) effects on life span.

**EXPERIMENT 3: MATING FREQUENCY, LIFETIME FECUNDITY, AND FEMALE SURVIVORSHIP WITH FOOD SUPPLEMENTATION**

When yeast and sugar–water were available to females, there were no significant differences in life span among treatments (Fig. 3b): mean life span was 23·5±1·0 days for once-mated females ($n=85$) and 22·0±1·0 days for multiply mated females ($n=86$).

![Graph showing egg production](image)

**Fig. 2.** Lifetime egg production of *Callosobruchus maculatus* females in experiment 2 (females mated once or multiply without yeast or sugar–water). (a) Lifetime fecundity against body size (elytron length). An analysis of covariance, with body size (elytron length) as the covariate, detected a significant effect of both treatment ($F_{1,226}=50·7$, $P<0·001$) and body size ($F_{1,226}=116·4$, $P<0·001$) on lifetime total egg production in both experiments. (b) Means±SE of the number of eggs laid in each 24-h period across the entire life. *Significant treatment differences ($P<0·05$) detected using a Mann–Whitney $U$-test comparing all surviving females at each age.
In many insect species, males have been demonstrated to provide nutrient benefits to females in their ejaculate (Boggs 1990). Male-derived substances have been detected in oocytes and/or female somatic tissue of many insect species (Friedel & Gillott 1977; Boggs & Gilbert 1979; Engebretson & Mason 1980; Boggs & Watt 1981; Greenfield 1982; Markow & Ankey 1984, 1988; Bownes & Partridge 1987; Butlin, Woodhatch & Hewitt 1987; Markow 1988; Markow, Gallagher & Krebs 1990; Pitnick, Markow & Riedy 1991), including two species of bruchid beetles (Huignard 1983; Boucher & Huignard 1987). For many species, however, the benefits of this transfer of nutrients during copulation have not been detectable (Greenfield 1982; Jones, Odendaal & Ehrlich 1986; Svard & Wiklund 1988; Oberhauser 1989; Wedell & Arak 1989), while in others, such as C. maculatus, benefits to the female are only detectable when females are nutrient stressed (Boucher & Huignard 1987; Butlin et al. 1987; Simmons 1988; Markow et al. 1990). These male-derived nutrients may improve female survivorship in C. maculatus by supporting metabolic functions during periods of nutrient stress.

(Mann–Whitney U-test, $U=3418.5$, $P=0.47$). In an analysis of covariance, with body size (elytron length) as a covariate, there were no significant treatment (mated once or mated multiply, $F_{1,168}=1.39$, $P=0.24$) or body size ($F_{1,168}=0.55$, $P=0.46$) effects on life span. Thus, the significant influence of both mating frequency and body size on life span, observed when females were starved (experiment 2), disappeared when yeast and sugar–water were available.

As in experiment 2, females in the multiply mated treatment laid more eggs than once-mated females (Fig. 4a), particularly at older ages (Fig. 4b). There were no significant among-treatment differences in body size (Mann–Whitney U-test, $U=3268$, $P=0.23$ for elytron length).

**Discussion**

For female C. maculatus, multiply mating increased average longevity by approximately 0–5 days when females were nutrient stressed (Fig. 3a), but had no effect on survivorship when females had access to yeast and sugar–water (Fig. 3b). This is consistent with the hypothesis that nutrients transferred during copulation are utilized by the female, potentially extending life span when nutrient stressed, but having little or no effect on life span when surplus food and water are available.
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or by being incorporated into eggs or somatic tissue, reducing the cost of egg production and somatic maintenance. To distinguish these hypotheses, more direct methods, such as radiolabel (Boggs & Gilbert 1979) or immunogenetic techniques (Friedel & Gilott 1977), are required.

Multiply mating also increased lifetime egg production of females in both experiments 2 and 3 (Figs. 2a and 4a). This is consistent with the hypothesis that female C. maculatus may require multiple matings to fertilize all of their eggs, as has been observed in many insect species (Ridley 1988). However, in these experiments I do not directly demonstrate that females are sperm depleted at older ages, because no information on the number of sperm remaining is available. The higher rate of egg production of multiply mated females could instead be due to a difference in egg maturation rates resulting from mechanical stimulation during mating (Boucher & Huignard 1987) or from the presence of spermatozoa or some component of the male accessory fluid in the female genital tract (Chen 1984).

A comparison of the results of experiments 2 and 3 suggests that females may not be sperm limited, but instead that females may decrease rates of egg laying while sperm are still available: females laid more eggs in both treatments of experiment 3 (with yeast and sugar–water available) than in the respective treatments of experiment 2 (with no yeast or sugar–water available). In the absence of yeast and sugar–water, female C. maculatus may not be able to continue vitellogenesis, and thus may not produce enough oocytes to utilize all the sperm they received during a single copulation. This has been observed in other species, where nutritionally deprived females are not capable of manufacturing enough oocytes to utilize all the sperm received during a single copulation (Boucher & Huignard 1987; Butlin et al. 1987; Markow et al. 1990). However, at least two alternative hypotheses may account for the among-experiment difference in egg production detected here: (1) females in experiment 2 may have metabolized stored sperm due to nutrient stress, and thus have no sperm remaining in storage; or (2) the observed difference may be an artefact of the three-generation gap between experiments 2 and 3 (although the two experiments were performed at different times, both were performed in the same incubator in the same environmental conditions).

The result that females confined with males did not produce more eggs than females mated only once is consistent with results for other species (e.g. Newport & Gromko 1984; Partridge et al. 1986; Partridge, Green & Fowler 1987). This reduction in egg production by C. maculatus females in the presence of males is unlikely due to increased mortality resulting from increased mating frequency (as discussed earlier), but may be accounted for by increased mortality or decreased oviposition rates due to male harassment (Wasserman 1986). Because mortality and behavioural data were not collected in this experiment, these hypotheses cannot be distinguished here.

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