

The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Received: 22 April 1993 / Accepted: 27 July 1993

Abstract. Maternal age influences offspring quality of many species of insects. This observed maternal age influence on offspring performance may be mediated through maternal age effects on egg size, which in turn may be directly influenced by the female's nutritional state. Thus, behaviors that influence a female's nutritional status will indirectly influence egg size, and possibly offspring life histories. Because males provide nutrients to females in their ejaculate, female mating frequency is one behavior which may influence her nutritional status, and thus the size of her eggs and the performance of her offspring. In this paper, I first quantify the influences of maternal age on egg size and offspring performance of the bruchid beetle, *Callosobruchus maculatus*. I then examine whether nutrients transferred during copulation reduce the magnitude of maternal age effects on egg size and larval performance when mothers are nutrient-stressed. Egg size and egg hatchability decreased, and development time increased, with increasing maternal age. Multiple mating and adult feeding by females both resulted in increased egg size. This increase in egg size of females mated multiply did not translate into reduced development time or increased body size and egg hatchability, but did correlate with improved survivorship of offspring produced by old mothers. Thus, it appears that because the influence of mating frequency on egg size is small relative to the influence of maternal age, the influence of nutrients derived from multiple mating on offspring life history is almost undetectable (detected only as a small influence on survivorship). For *C. maculatus*, female multiple mating has been demonstrated to increase adult female survivorship (Fox 1993a), egg production (Credland and Wright 1989; Fox 1993a), egg size, and larval survivorship, but, contrary to the suggestion of Wasserman and Asami (1985), multiple mating had no detectable influence on offspring development time or body size.

Key words: Life history – Maternal effects – Multiple mating – Nutrient-stress

Maternal age influences offspring quality of many species of insects (Parsons 1964; Mousseau and Dingle 1991). Offspring of older mothers frequently suffer higher mortality, develop slower, and are smaller at adult emergence than offspring from younger mothers (Mousseau and Dingle 1991). Similarly, older mothers frequently lay smaller eggs (Jones et al. 1982; Wiklund and Persson 1983), such that much of the observed maternal age influence on offspring performance may be mediated through egg size (Mousseau and Dingle 1991).

The influence of female age on egg size, and thus offspring performance, may be mediated by the female's nutritional state. Although female insects generally emerge as adults with numerous eggs already matured and ready to oviposit (Wilson and Hill 1989), in many species females subsequently mature more eggs, which are either oviposited or stored in the ovaries and later resorbed (Engelmann 1970; Wilson and Hill 1989). Due to the gradual exhaustion of the female's resources, the eggs that are matured post-emergence may be smaller, poorer quality eggs than eggs matured pre-emergence, particularly when the females are nutrient-stressed. This difference in egg size and quality may then translate into differences in offspring mortality, development rate, and body size (Murphy et al. 1983; Rossiter 1991).

If a decline in egg size is mediated by a female's nutritional status, then behaviors that influence nutritional status will indirectly influence egg size, and possibly offspring life histories. In many insect species, males provide nutrient benefits to females in their ejaculate (Boggs 1990). Male derived substances have been detected in oocytes and female somatic tissue of many species (Boggs 1990), including two species of bruchid beetles (Huignard 1983; Boucher and Huignard 1987). For many species, however, the benefits of this transfer of nutrients during copulation have not been detectable

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(Greenfield 1982; Jones et al. 1986; Svard and Wiklund 1988; Oberhauser 1989; Wedell and Arak 1989), while in others benefits to the female have been detected only when females were nutrient-stressed (Gwynne 1984, 1988; Gwynne et al. 1984; Boucher and Huignard 1987; Butlin et al. 1987; Simmons 1988; Markow et al. 1990; Fox 1993a).

In the bruchid beetle, *C. maculatus*, multiple mating increases average female longevity by approximately 0.5 days when females are nutrient-stressed (starved), but has no effect on survivorship when females have unlimited access to yeast and sugar-water (Fox 1993a). This result is consistent with the hypothesis that nutrients transferred during copulation are utilized by female *C. maculatus* for somatic maintenance and egg production when nutrient-stressed, but that the male contribution is relatively unimportant when surplus food and water are available.

In this paper, I first quantify a male's potential contribution to egg production by measuring ejaculate sizes of the bruchid beetle, *Callosobruchus maculatus*. I then quantify the influence of maternal age on egg size and offspring performance. Finally, I extend my investigation of the influence of multiple mating on the life-history of *C. maculatus* (Fox 1993a) by examining whether nutrients transferred during copulation reduce the magnitude of maternal age effects on egg size and offspring performance under conditions of nutrient-stress, as has been suggested by Wasserman and Asami (1985).

Methods and materials

Natural history

Callosobruchus maculatus is a cosmopolitan pest of stored legumes (Fabaceae). Females generally mate multiple times during their lifetime, although only one, or occasionally two, matings are required to fertilize all of their eggs (C. Fox, unpublished data; P. Eady, personal communication). Following mating, females commence egg-laying (often within minutes, if seeds are available), cementing their eggs to the surface of the host seeds (Messina 1991; Fox 1993b). Approximately 4–5 days later (at 28°C), the eggs hatch and the first instar larvae burrow into the seed. Larval development and pupation are completed entirely within a single seed.

Populations of *C. maculatus* are most commonly detected in stores of dried legumes. These beetles have likely been associated with dried legumes for thousands of years (Messina 1991), and their life cycle appears well adapted for reproduction in a storage environment. Reproduction without access to food and water is typical for adults in storage conditions. Although adults will readily feed on yeast and sugar-water in the laboratory, which increase survivorship and egg production of adult females (Fox 1993a), emerging adults require neither food nor water to reproduce.

All beetles used in these experiments were collected from stored azuki beans (*Vigna angularis*) near San Francisco, California (Fox 1993b). The laboratory population was established with more than 1000 eggs and maintained on azuki beans, before and during the experiment, at 27 ± 1°C, 24 h light. Beetles from approximately the 10th laboratory generation were used in the experiments presented here.

Male ejaculate size

A male's potential contribution to egg production was estimated by measuring the size of male ejaculates. Ejaculate size was estimated

by comparing both male and female body weight before and after mating. For each estimate of body weight, an individual was weighed twice to 0.01 mg precision on an electronic balance. The estimate of the individual's body weight was the average of these two weighings. If both weighings differed by more than 0.04 mg, a third weighing was performed, and the estimate of body weight was then the average of these three weighings. Ejaculate size was calculated as (weight gain of female + weight loss of male)/2. This technique is moderately precise: male weight loss during mating was highly correlated with female weight gain during mating (Pearson correlation $r = 0.76$; $P < 0.001$).

Maternal age and mating frequency influences on egg size and offspring performance

The following experiment was designed to quantify the influences of maternal age and mating frequency on egg size and offspring performance. Virgin females, collected from isolated seeds within 12 h of adult emergence from the seed, were presented with a single virgin male in a 30 mm petri dish and allowed to copulate. Following a single copulation, females were transferred to 60 mm petri dishes containing approximately 3.5 g dry azuki seeds and allowed to oviposit. These females were then divided into four treatments: (1) Mated once, nutrient-stressed; (2) Mated multiply, nutrient-stressed; (3) Mated once, supplemental diet; (4) Mated multiply, supplemental diet.

The two groups of experimental females in the supplemental diet treatments were provided with baker's yeast (Red Star active dry yeast) *ad libitum* and a 5% sucrose solution (supplied in 0.5 dram shell vials stoppered with cotton and replaced every 48 h throughout the experiment). Females in the two nutrient-stressed treatments were maintained without access to yeast or sugar water.

Females in the two multiply mated treatments were remated every 48 h. These females were transferred into an empty 30 mm petri dish, presented with one virgin male less than 12 h old, and allowed to copulate once (as in Fox 1993b). These females were then transferred into a new 60 mm petri dish containing 3.5 g azuki seeds. All females were maintained solitarily between matings. The females in the once-mated treatments were mated only once, immediately following adult emergence.

Every 12 h (until death) each female in each treatment was transferred to a new dish containing 3.5 g dry azuki seeds. Length and width of eggs from each half day were measured using an optical micrometer on a 50× dissecting scope. Because the benefits of nutrients transferred by males during copulation may be detectable only when females are nutrient-stressed (see introduction), eggs from the two nutrient-stressed treatments (those where females had no access to yeast or sugar-water) were reared to adult. Beetles were reared at densities of one beetle per azuki seed. Emerging adults were collected and weighed individually within 12 h of adult emergence. Eggs from age-classes 1.5, 2, 3.5, and 4 days, however, were not measured or reared to adult. Data on egg size were thus collected for all four treatments, while data on larval survivorship, development time, and body size were collected in only the two nutrient-stressed treatments.

Data on egg hatching success (proportion of eggs hatching) were recorded in a nearly identical experiment performed four months earlier with the same laboratory population. This other experiment was identical to the above described experiment except that (1) eggs were collected at 24 h intervals rather than 12 hour intervals, and (2) eggs of 115 females were collected in each treatment (rather than 40 females per treatment) (Fox 1993b for details).

Because females were transferred to new seeds at 12 hour intervals, female ages will be divided into age-classes. For statistical analyses of egg size, development time, and body size, an age-class is a 12 h period, in which eggs laid in an age-class are laid in the twelve h previous to that day (e.g. eggs laid at age-class 0.5 days are those laid between adult emergence and 12 h post-emergence, and eggs laid at age-class 6 days are eggs laid between 5.5 and 6 days

post-emergence). For analyses of egg hatchability and larval survivorship, age-classes are 24 hour periods (e.g. eggs laid in age-class 1 day were laid in the first 24 h post-emergence).

Data used in all statistical tests are the average egg size, survivorship, development time, or body size of offspring produced by a female during each age-class. All multiple comparisons tests follow the sequential bonferroni procedure as described by Rice (1989).

Results

Male ejaculate size

Males contributed an average of 0.18 ± 0.01 mg of material to females in their first mating post-emergence ($n=42$). This was equivalent to 5.1 ± 0.2 percent of the average male weight at emergence, and 3.3 ± 0.2 percent of the average female weight at emergence. In the multiply-mated treatments of the following experiments, each female mated with 3 to 5 virgin males, for an estimated male contribution of between 0.54 and 0.90 mg, which is 9.9 to 16.5 percent of the female's body weight at emergence.

Maternal age and size both influence egg size

For all age-classes, larger females laid larger eggs (Fig. 1). Within age-class 0.5 days (eggs laid in the first 12 h post-emergence), in which most eggs that are laid have been matured pre-eclosion (Wilson and Hill 1989), female body weight was positively correlated with egg length ($P < 0.01$, $n=115$), but not with egg width ($P=0.12$) (Fig. 1).

In all four treatments, egg width decreased as females aged (Table 1). Females with access to yeast and sugar-

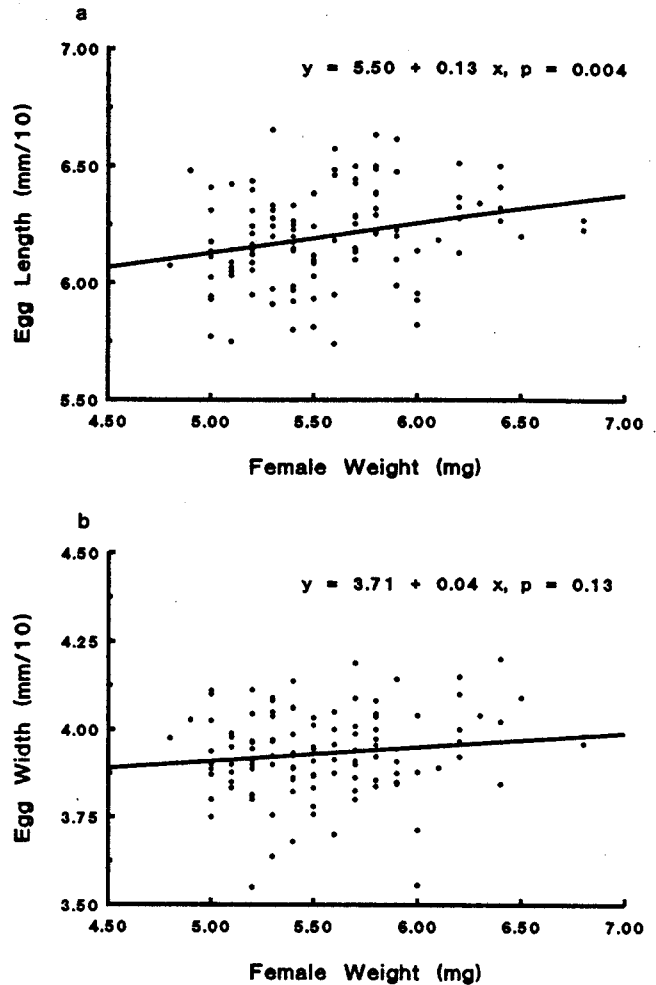


Fig. 1a, b. The influence of female body size on the egg size of *Callosobruchus maculatus*. Only eggs laid in the first 12 h post-emergence are included in this analysis. a Egg length; b Egg width

Table 1. Mean egg widths (mm/10) for females in four treatments and 13 age-classes. Mean egg size was calculated as the average (across females) of the average egg size (across all eggs laid by a single female during that half day). Number of females contributing to the estimate is in parentheses. All age-classes represent half day periods (e.g. age-class 4.5 represents eggs laid between age 4.0 days

and 4.5 days). Type III sums-of-squares were calculated for the 2-way analyses of variance using SAS GLM. * indicate significance following a sequential bonferroni (Rice 1989; number of comparisons = 13 for each main effect). When an interaction between mating treatment and feeding treatment was included in the model, all interaction effects were non-significant

Age-Class	Mated Once		Mated Multiply		2-way Analyses of Variance			
	w/o food	w/ food	w/o food	w/ food	Food Treatment		Mating Treatment	
					F	P	F	P
0.5	3.93 (19)	3.95 (20)	3.96 (20)	3.94 (20)	0.01	ns	0.17	ns
1.0	3.76 (19)	3.84 (20)	3.86 (20)	3.84 (18)	0.99	ns	3.11	ns
2.5	3.69 (19)	3.78 (20)	3.73 (20)	3.79 (20)	9.00	0.00*	1.12	ns
3.0	3.66 (19)	3.76 (18)	3.66 (20)	3.79 (19)	23.44	0.00*	0.65	ns
4.5	3.55 (18)	3.66 (20)	3.60 (20)	3.74 (20)	23.37	0.00*	7.71	0.01
5.0	3.48 (18)	3.62 (19)	3.53 (20)	3.70 (20)	27.78	0.00*	4.72	0.03
5.5	3.37 (17)	3.59 (18)	3.49 (20)	3.69 (19)	46.73	0.00*	13.77	0.00*
6.0	3.37 (15)	3.54 (19)	3.46 (19)	3.65 (18)	29.11	0.00*	9.05	0.00*
6.5	3.30 (12)	3.51 (17)	3.41 (17)	3.61 (20)	37.26	0.00*	10.13	0.00*
7.0	3.25 (11)	3.46 (19)	3.36 (12)	3.59 (18)	22.36	0.00*	8.01	0.01
7.5	3.29 (8)	3.48 (19)	3.44 (10)	3.57 (19)	10.97	0.00*	6.06	0.02
8.0	3.12 (3)	3.49 (17)	3.32 (9)	3.59 (19)	31.58	0.00*	6.09	0.02
8.5	3.15 (5)	3.46 (18)	3.34 (7)	3.54 (18)	6.12	0.02	0.86	ns

- df for all treatment effects is 1

water laid larger eggs at older ages than nutrient-stressed females, and females that mated multiply laid larger eggs at older ages than females that mated only once (two-way analyses of variance, Table 1). When a feeding treatment \times mating treatment interaction was included in the analyses of variance, this interaction was not significant for any age-class, suggesting that the influences of both feeding and mating treatments on egg size were additive. Thus, contrary to expectations, multiple mating resulted in larger eggs for *both* females that were nutrient-stressed and females that had access to yeast and sugar-water *ad libitum*.

The maternal age influence on egg length was similar to the influence on egg width: eggs of older mothers were shorter than eggs of younger mothers (data not presented here). However, the influence of both feeding and mating treatments on egg length were less striking: although the general patterns were the same, in a two-way analysis of variance (as in Table 1) the feeding treatment effect was not significant for any age-class, while the mating treatment effect was significant for only age-class 6.5 days.

Thus, although both feeding treatment and mating treatment had a large effect on egg width, they had no statistically detectable effect on egg length.

Maternal age and offspring performance

Only offspring of females from the two nutrient-stressed treatments were reared to adult.

Offspring of older mothers took significantly longer to develop than offspring of young mothers (Fig. 2). In both once-mated and multiply-mated treatments, for both sexes of offspring, eggs laid in the first half-day (age-class 0.5 days) developed significantly faster than eggs laid by the same female at all older ages, with the exception of age-class 1 day, which did not differ from age-class 0.5 day for either sex or treatment (Wilcoxon Signed-Rank Test, corrected for multiple comparisons, with 9 comparisons for each treatment and sex; $P < 0.05$).

Offspring from both young and old mothers were approximately the same size at adult emergence (Fig. 3).

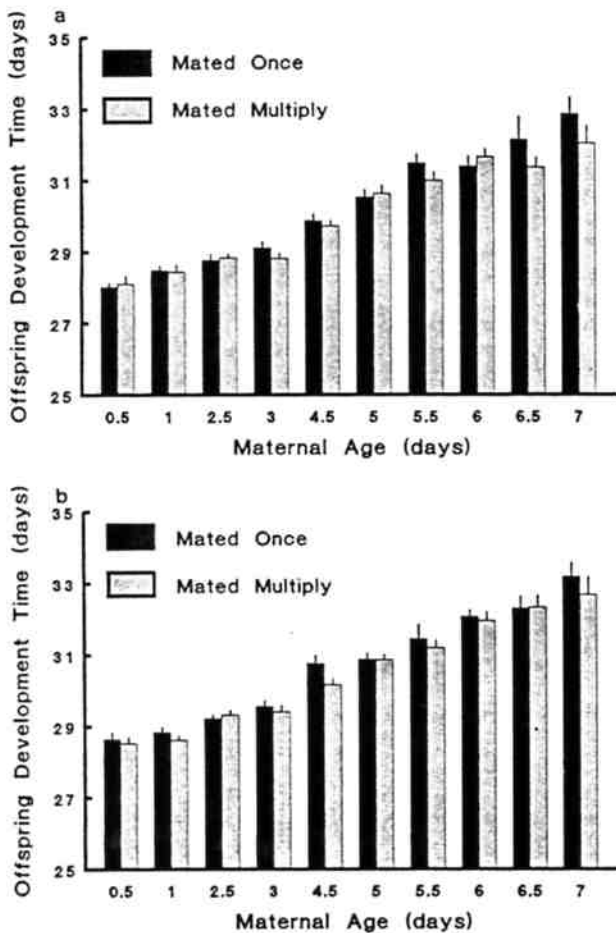


Fig. 2a, b. The influence of maternal age on development time of her a male and b female offspring. *Callosobruchus maculatus* offspring reared from eggs laid by young mothers developed significantly faster than offspring reared from eggs laid by the same mothers when they were older (in a one-way repeated measures analysis of variance, the effect of maternal age was significant for both treatments and both sexes; $P < 0.001$).

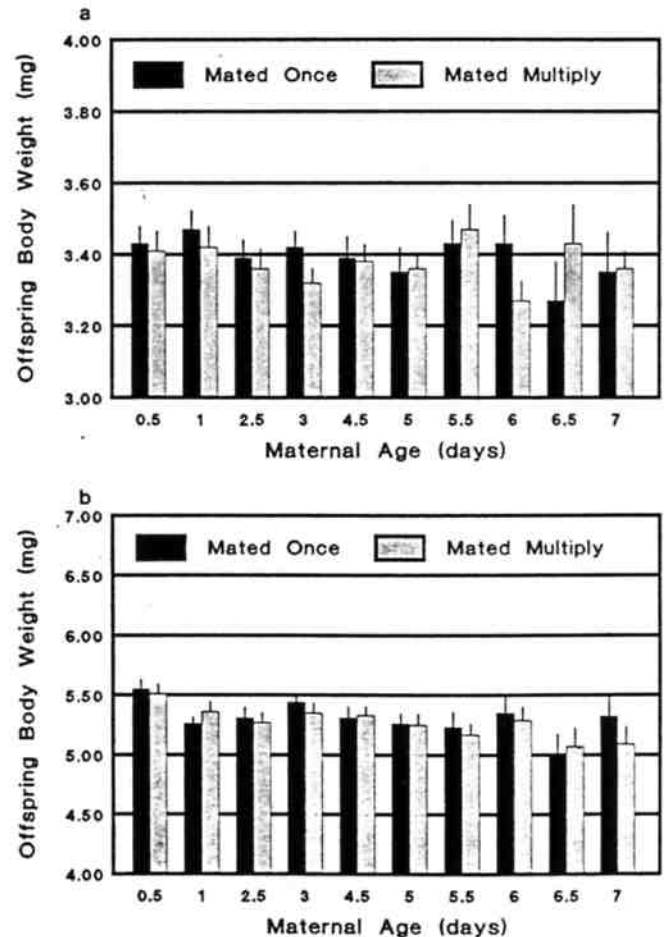


Fig. 3a, b. The influence of maternal age on body size of her a male and b female offspring. *Callosobruchus maculatus* offspring reared from eggs laid by young mothers developed to be approximately the same size as offspring reared from eggs laid by the same mothers when they were older (in a one-way repeated measures analysis of variance, the effect of maternal age was not significant for either treatment or sex; $P > 0.05$).

For neither sex in either treatment did eggs laid the first half-day (age-class 0.5 day) produce larger offspring than eggs laid by the same female in any other age-class (Wilcoxon Signed-Rank Test corrected for multiple comparisons, with 9 comparisons for each treatment and sex; $P < 0.05$). However, a two-way repeated measures analysis of variance (Wilkinson 1990), with mating treatment as a categorical variable and maternal age-class as the repeated measure, detected a significant influence of maternal age on female offspring size ($P < 0.01$), but not on male offspring size ($P > 0.50$). Thus, there appears to be a trend toward older females producing smaller female offspring (Fig. 3b), but the influence of maternal age on offspring size is relatively small (e.g. in the one-way repeated measures analyses of variance, the effect of maternal age was not significant for either treatment or sex).

Both egg hatchability (the proportion of eggs which hatched) and larval survivorship decreased with increasing maternal age (Fig. 4). Eggs laid in age-classes 5 and 6 for once mated females, and eggs laid in age-classes 5 through 9 by multiply mated females, were less likely to hatch than eggs laid in age-class 1 (Fig. 4a; Wilcoxon Signed-Rank Test corrected for multiple comparisons,

with 8 comparisons for each treatment; $P < 0.05$). For larval survivorship, no age-classes differed significantly from age-class 1 (Wilcoxon Signed-Rank Test corrected for multiple comparisons, with 6 comparisons for each treatment), although there was a trend for offspring of older females to have much lower survivorship in each treatment (Fig. 4b).

Because egg size dramatically decreases with maternal age, much of the maternal age influence on offspring performance observed above may be mediated through the maternal age effect on egg size (Mousseau and Dingle 1991). In this experiment it is not possible to separate the effects of increasing maternal age from decreasing egg size, so we can not directly test the influence of egg size. However, within age-class 0.5 (in which egg size varies across females, independent of female age), there was no significant relationship between egg length or egg width and either offspring development time, body size (least-squares linear regression, $P > 0.05$ for each sex), or larval survivorship (Pearson correlation, $P > 0.05$), suggesting that egg size does not influence subsequent larval performance. Because eggs are glued directly to host seeds, it is not possible to examine the influence of egg volume or egg weight on offspring performance.

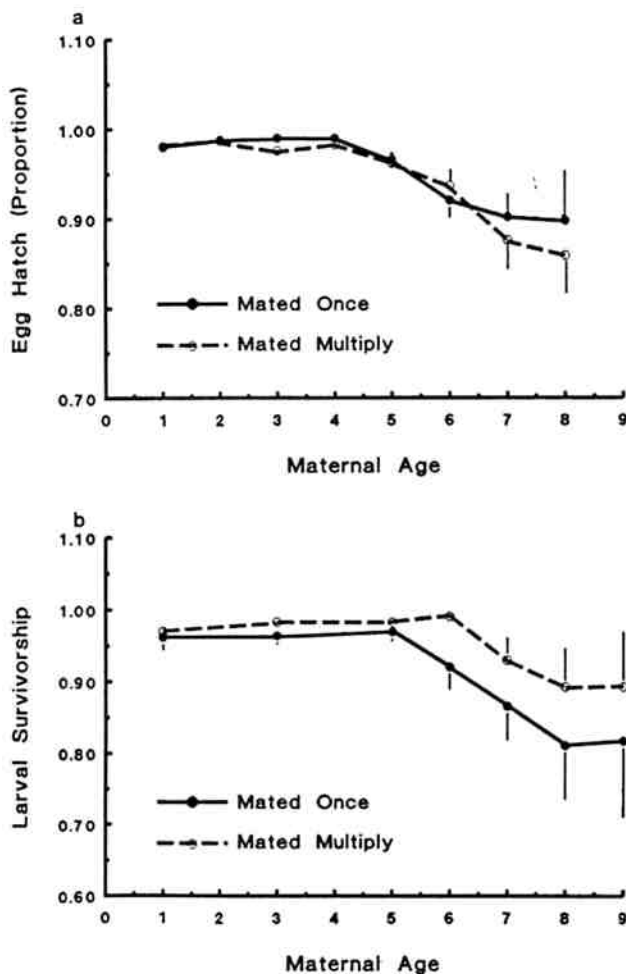


Fig. 4. The influence of maternal age on a egg hatchability and b larval survivorship of *Callosobruchus maculatus*

Mating frequency and offspring performance

Mating treatment (once-mated or multiply-mated) had no detectable effect on development time or body size of offspring, or the hatchability of eggs laid by females in any age-class (Mann-Whitney U-test for each age-class, corrected for multiple comparisons, 10 comparisons in each group) (Figs. 2, 3 and 4a). Thus, although multiple mating resulted in increased egg size of older females, this increase in egg size did not translate into shorter development time nor increased body size of the offspring reared from these eggs.

Mating treatment did influence larval survivorship (Fig 4b): larval survivorship was higher in the multiply mated treatment for all age-classes older than age 1 day, although larval survivorship differed significantly between treatments only for age-class 6.0 days (Mann-Whitney U-tests, corrected for multiple comparisons, 7 comparisons in each group; $P < 0.05$).

Discussion

For *Callosobruchus maculatus*, egg size and egg hatchability decreased (Table 1 and Fig. 4a), and development time increased (Fig. 2), with increasing maternal age. Both multiple mating and adult feeding by females resulted in increased egg size. The increase in egg size resulting from multiple mating of nutrient-stressed females did not translate into reduced development time or increased body size and egg hatchability, but did correlate with improved survivorship of offspring produced by old mothers. Thus, it appears that because the influence of mating frequency on egg size is small relative to the influence of maternal age (Table 1), the influence of

nutrients derived from multiple mating on offspring life history is very small (only detectable as a weak effect on survivorship; Figs. 2–4). For *C. maculatus*, female multiple mating has been demonstrated to increase maternal survivorship (Fox 1993a), lifetime fecundity (Credland and Wright 1989; Fox 1993a), egg size, and offspring survivorship, but, contrary to the suggestion of Wasserman and Asami (1985), multiple mating had no detectable influence on offspring development time or body size.

As with *C. maculatus*, egg size has generally been reported to decrease with maternal age in other insects (Leonard 1970; Harvey 1977; Jones et al. 1982; Murphy et al. 1983; Wiklund and Persson 1983; Yuma 1984; Karlsson and Wiklund 1984, 1985; Wasserman and Asami 1985; Boggs 1986; Svard and Wiklund 1988; but see Moore and Singer 1987), although both an increase (Kasule 1991) or no change in egg size (Berger 1989; Marshall 1990) have also been reported. This decrease in egg size is generally attributed to a depletion of the female's resources, such that later laid eggs are necessarily smaller and/or of poorer quality (Richards and Myers 1980; Wiklund and Karlsson 1984). Alternatively, Begon and Parker (1986) have proposed that decreasing egg size with increasing maternal age may be adaptive when female clutch size is constrained. Although the experiment reported here was not designed to sort among these hypotheses, the result that females with access to food laid larger eggs than nutrient-stressed or once-mated females, respectively, is consistent with the "resource depletion" hypothesis. Maternal feeding has also been reported to increase egg size in other insects (Harvey 1983; Murphy et al. 1983; Steinwascher 1984; Wallin et al. 1992), likewise supporting the "resource depletion" hypothesis.

Also consistent with the "resource depletion" hypothesis was the result that female *C. maculatus* that were mated multiply laid larger eggs than females that were mated only once. This result suggests that male-derived substances are being incorporated into female's oocytes. Male-derived substances have been detected in oocytes and/or female somatic tissue of many other insect species (Friedel and Gillott 1977; Boggs and Gilbert 1979; Engbretson and Mason 1980; Mullins and Keil 1980; Boggs and Watt 1981; Greenfield 1982; Schal and Bell 1982; Bowen et al. 1984; Markow and Ankey 1984, 1988; Bownes and Partridge 1987; Butlin et al. 1987; Sivinski and Smittle 1987; Markow 1988; Markow et al. 1990; Pitnick et al. 1991), including two other species of bruchid beetles (Huignard 1983; Boucher and Huignard 1987). Multiple mating has been found to result in increased egg size in crickets and katydids (Gwynne 1984, 1988; Simmons 1988), but had no influence on egg size in the monarch butterfly (Svard and Wiklund 1988). For the above crickets and katydids, however, the benefits of multiple mating have only been detectable under conditions of nutrient stress (Gwynne 1988; Simmons 1988). For *C. maculatus*, multiple mating resulted in increased egg size both when females were nutrient-stressed and when females had unlimited access to yeast and sugar-water. In addition, the effects of mating frequency and

feeding treatment appeared to be additive (based on a non-significant interaction term in the analyses of variances), suggesting that the male contribution to egg production supplements the nutrients available from yeast and sugar-water, possibly by providing some limiting nutrient not available in the yeast and sugar-water. Alternatively, males may be transferring some accessory gland secretion which influences egg production (Chen 1984).

The observed decrease in egg size with increasing maternal age appears to translate into decreased offspring performance in *C. maculatus*: egg hatchability decreased, and the development time of offspring increased, with maternal age. It is likely that these patterns are at least partially a result of the smaller eggs laid by older females, possibly interacting with a change in egg quality. In this experiment, however, egg size is not independent of maternal age, so it is not possible to test whether egg size is directly responsible for the observed maternal age effect on egg hatchability and development time.

Because egg size varies across females, it is possible to examine the influence of egg size on offspring performance by examining the influence of among-female variation in egg size (controlling for maternal age) on among-family variation in offspring performance. A positive correlation between egg size and offspring performance among females of the same age class would suggest that within female variation in offspring performance may be partially mediated by egg size. However, for *C. maculatus* there was no relationship between egg width or egg length and any performance character of the offspring.

This absence of a correlation between egg size and offspring performance in *C. maculatus* is unusual for insects: offspring developing from larger eggs generally hatch earlier (Rossiter 1991; Wallin et al. 1992), have higher hatching success (Richards and Myers 1980; Simmons 1988), higher larval survivorship (Wallin et al. 1992), develop faster (Steinwascher 1984; Yuma 1984; Rossiter 1991), develop into larger adults or pupae (Steinwascher 1984; Yuma 1984; Honek 1987; Rossiter 1991), breed earlier (Sibly and Monk 1987), and/or have higher starvation tolerance (Solbreck et al. 1989; Carlberg 1991; Tauber et al. 1991; but see Lamb and Smith 1980) or desiccation tolerance (Sota and Mogi 1992). However, some studies have failed to detect an effect of egg size on survivorship (Wiklund and Persson 1983; Steinwascher 1984; Karlsson and Wiklund 1984; Wiklund and Karlsson 1984), development rate (Wiklund and Persson 1983; Karlsson and Wiklund 1984; Wiklund and Karlsson 1984), offspring body size (Richards and Myers 1980; Wiklund and Persson 1983; Karlsson and Wiklund 1984; Wiklund and Karlsson 1984), or egg mortality (Wiklund and Persson 1983; Karlsson and Wiklund 1984, 1985; Wiklund and Karlsson 1984). One likely explanation for the absence of a positive egg size – offspring performance correlation in *C. maculatus* may be that larvae were reared at low density on a uniformly high quality resource (commercial azuki beans, *Vigna angularis*). As found in many studies, variation in egg

size may be of significance only when offspring are reared under adverse conditions, such as low quality or quantity of a resource. Thus, we are in need of substantially more data concerning the egg size on offspring performance before we can generalize and ask the very interesting question: If egg size is negatively correlated with egg number, then why don't females lay more small eggs rather than fewer large eggs?

Although a large maternal age effect was detected for both development time and egg hatchability of *C. maculatus*, no maternal age influence was detected on offspring body size. This absence of an effect on offspring body size indicates that offspring developing from later laid eggs may be compensating for small egg size by developing longer to eventually pupate at the same size as offspring developing from earlier laid eggs. This suggests that size, rather than development time, may be a cue as to when larvae should pupate. Similar results have been found in other systems. For example, *Battus philenor* larvae reared on low quality diets take longer to develop, but pupate at approximately the same size, suggesting that size is a cue as to when to pupate (Tatar 1986).

Acknowledgements. I am very grateful to R. Caldwell and H. Dingle for providing laboratory space. J. Rosenheim provided the microbalance used to weigh many thousands of beetles. Financial support was provided by the Department of Integrative Biology at UC Berkeley, and by an NIH Training Grant in Genetics. I am also grateful to R. Caldwell, S. Carroll, H. Dingle, L. McLennan, C. Peterson, T. Prout, M. Tatar and two anonymous reviewers for helpful comments or discussion concerning these experiments and/or this manuscript.

References

- Begon M, Parker GA (1986) Should egg size and clutch size decrease with age? *Oikos* 47:293-302
- Berger A (1989) Egg weight, batch size and fecundity of the spotted stalk borer, *Chilo partellus* in relation to weight of females and time of oviposition. *Entomol Exp Appl* 50:199-207
- Boggs CL (1986) Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecol Entomol* 11:7-15
- Boggs CL (1990) A general model of the role of male-donated nutrients in female insects' reproduction. *Am Nat* 136:598-617
- Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* 206:83-84
- Boggs CL, Watt WB (1981) Population structure of pierid butterflies. IV. Genetic and physiological investment in offspring by male *Colias*. *Oecologia* 50:320-324
- Boucher L, Huignard J (1987) Transfer of male secretions from the spermatophore to the female insect in *Caryedon serratus* (Ol.): analysis of the possible trophic role of these secretions. *J Insect Physiol* 33:949-957
- Bowen BJ, Codd CG, Gwynne DT (1984) The katydid spermatophore (Orthoptera: Tettigoniidae): male nutritional investment and its fate in the female. *Aust J Zool* 32:23-31
- Bowen BJ, Partridge L (1987) Transfer of molecules from ejaculate to females in *Drosophila melanogaster* and *Drosophila pseudoobscura*. *J Insect Physiol* 12:941-947
- Butlin RK, Woodhatch CW, Hewitt GM (1987) Male spermatophore investment increases female fecundity in a grasshopper. *Evolution* 41:221-225
- Carlberg U (1991) Egg-size variation in *Extatosoma tiaratum* (MacLeay) and its effect on survival and fitness of newly hatched nymphs (Insecta; Phasmida). *Biol Zentral bl* 110:163-173
- Chen PS (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. *Annual Review of Entomology* 29:233-255
- Credland PF, Wright AW (1989) Factors affecting female fecundity in the cowpea seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J Stored Prod Res* 25:125-136
- Engelbreton JA, Mason WH (1980) Transfer of ^{65}Zn at mating in *Heliothis virescens*. *Environ Entomol* 9:119-121
- Engelmann F (1970) *The Physiology of Insect Reproduction*. Pergamon Press, Oxford
- Fox CW (1993a) Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera, Bruchidae). *Funct Ecol* 7:203-208
- Fox CW (1993b) A quantitative genetic analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus*. *Evolution* 47:166-175
- Friedel T, Gillott C (1977) Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. *J Insect Physiol* 23:145-151
- Greenfield MD (1982) The question of paternal investment in Lepidoptera: male-contributed proteins in *Plodia interpunctella*. *Inter J Inverte Repro* 5:323-330
- Gwynne DT (1984) Courtship feeding increases female reproductive success in bushcrickets. *Nature* 307:361-363
- Gwynne DT (1988) Courtship feeding and the fitness of female katydids (Orthoptera: Tettigoniidae). *Evolution* 42:545-555
- Gwynne DT, Bowen BJ, Codd CG (1984) The function of the katydid spermatophore and its role in fecundity and insemination (Orthoptera: Tettigoniidae). *Aust J Zool* 32:15-22
- Harvey GT (1977) Mean weight and rearing performance of successive egg clusters of eastern spruce budworm (Lepidoptera: Tortricidae). *Can Ent* 109:487-496
- Harvey GT (1983) Environmental and genetic effects on mean egg weight in spruce budworm (Lepidoptera: Tortricidae). *Can Ent* 115:1109-1117
- Honek A (1987) Regulation of body size in a heteropteran bug, *Pyrrhocoris apterus*. *Entomol Exp Appl* 44:257-262
- Huignard J (1983) Transfer and fate of male secretions deposited in the spermatophore of females of *Acanthoscelides obtectus* Say (Coleoptera Bruchidae). *J Insect Physiology* 29:55-63
- Jones KN, Odendaal FJ, Ehrlich PR (1986) Evidence against the spermatophore as paternal investment in the checkerspot butterflies (Euphydryas: Nymphalidae). *Am Midl Nat* 116:1-6
- Jones RE, Hart JR, Bull GD (1982) Temperature, size and egg production in the cabbage butterfly, *Pieris rapae* L. *Aust J Zool* 30:223-232
- Karlsson B, Wiklund C (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly *Lasiommata megera*. *Oikos* 43:376-385
- Karlsson B, Wiklund C (1985) Egg weight variation in relation to egg mortality and starvation endurance of newly hatched larvae in some satyrid butterflies. *Ecol Entomol* 10:205-211
- Kasule FK (1991) Egg size increases with maternal age in the cotton stainer bugs *Dysdercus fasciatus* and *D. cardinalis* (Hemiptera: Pyrrhocoridae). *Ecol Entomol* 16:345-349
- Lamb RJ, Smith SM (1980) Comparisons of egg size and related life-history characteristics for two predaceous tree-hole mosquitos (*Toxorhynchites*). *Can J Zool* 58:2065-2070
- Leonard DE (1970) Intrinsic factors causing qualitative changes in populations of *Porthetria dispar* (Lepidoptera: Lymantriidae). *Can Ent* 102:239-249
- Markow TA (1988) *Drosophila* males provide a material contribution to offspring sired by other males. *Funct Ecol* 2:77-79
- Markow TA, Ankney PF (1984) *Drosophila* males contribute to oogenesis in a multiple mating species. *Science* 224:302-303
- Markow TA, Ankney PF (1988) Insemination reaction in *Drosophila*: found in species whose males contribute material to oocytes before fertilization. *Evolution* 42:1097-1101

- Markow TA, Gallagher PD, Krebs RA (1990) Ejaculate-derived nutritional contribution and female reproductive success in *Drosophila mojavensis* (Patterson and Crow). *Funct Ecol* 4: 67–73
- Marshall LD (1990) Intraspecific variation in reproductive effort by female *Parapediasia teterella* (Lepidoptera: Pyralidae) and its relation to body size. *Can J Zool* 68: 44–48
- Messina FJ (1991) Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. *Oecologia* 85: 447–455
- Moore RA, Singer MC (1983) Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas editha*. *Ecol Entomol* 12: 401–408
- Mousseau TA, Dingle H (1991) Maternal effects in insect life histories. *Annu Rev Entomol* 36: 511–534
- Mullins DE, Keil CB (1980) Paternal investment of urates in cockroaches. *Nature* 283: 567–569
- Murphy DD, Launer AE, Ehrlich PR (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. *Oecologia* 56: 257–263
- Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. *Behav Ecol Sociobiol* 25: 237–246
- Parsons PA (1964) Parental age and the offspring. *Q Rev Biol* 39: 258–275
- Pitnick S, Markow TA, Riedy MF (1991) Transfer of ejaculate and incorporation of male-derived substances by females in the Nannoptera species group (Diptera: Drosophilidae). *Evolution* 45: 774–780
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43: 203–208
- Richards LJ, Myers JH (1980) Maternal influences on size and emergence time of the cinnabar moth. *Can J Zool* 58: 1452–1457
- Rossiter MC (1991) Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. *Funct Ecol* 5: 386–393
- Schal C, Bell WJ (1982) Ecological correlates of paternal investment of urates in a tropical cockroach. *Science* 218: 170–173
- Sibly R, Monk K (1987) A theory of grasshopper life cycles. *Oikos* 48: 186–194
- Simmons LW (1988) The contribution of multiple mating and spermatophore consumption to the lifetime reproductive success of female field crickets (*Gryllus bimaculatus*). *Ecol Entomol* 13: 57–69
- Sivinski J, Smittle B (1987) Male transfer of materials to mates in the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Flor Entomol* 70: 233–238
- Solbreck C, Olsson R, Anderson DB, Forare J (1989) Size, life history and response to food shortage in two geographical strains of the seed bug *Lygaeus equestris*. *Oikos* 55: 387–396
- Sota T, Mogi M (1992) Interspecific variation in desiccation survival time of *Aedes (Stegomyia)* mosquito eggs is correlated with habitat and egg size. *Oecologia* 90: 353–358
- Steinwascher K (1984) Egg size variation in *Aedes aegypti*: relationship to body size and other variables. *Am Midl Nat* 112: 76–84
- Svard L, Wiklund C (1988) Fecundity, egg weight and longevity in relation to multiple matings in females of the monarch butterfly. *Behav Ecol Sociobiol* 23: 39–43
- Tatar M (1986) The induction of diapause in the pipevine swallowtail, *Battus philenor*. Masters thesis, Dept. Zoology, University of California, Davis, CA, USA
- Tauber CA, Tauber MJ, Tauber MJ (1991) Egg size and taxon: their influence on survival and development of chrysopterid hatchlings after food and water deprivation. *Can J Zool* 69: 2644–2650
- Wallin H, Chiverton PA, Ekblom BS, Borg A (1992) Diet, fecundity and egg size in some polyphagous predatory carabid beetles. *Entomol Exp Appl* 65: 129–140
- Wasserman SS, Asami T (1985) The effect of maternal age upon fitness of progeny in the southern cowpea weevil, *Callosobruchus maculatus*. *Oikos* 45: 191–196
- Wedell N, Arak A (1989) The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). *Behav Ecol Sociobiol* 24: 117–125
- Wiklund C, Karlsson B (1984) Egg size variation in satyrid butterflies: adaptive vs. historical, “Bauplan”, and mechanistic explanations. *Oikos* 43: 391–400
- Wiklund C, Persson B (1983) Fecundity, and the relation of egg weight to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? *Oikos* 40: 53–63
- Wilkinson L (1990) Systat: The system for statistics. Systat, Inc., Evanston, IL
- Wilson K, Hill L (1989) Factors affecting egg maturation in the bean weevil *Callosobruchus maculatus*. *Physiol Entomol* 14: 115–126
- Yuma M (1984) Egg size and variability of the firefly, *Luciola cruciata* (Coleoptera, Lampyridae). *Kontyu* 52: 615–629