Paternal Investment in a Seed Beetle (Coleoptera: Bruchidae): Influence of Male Size, Age, and Mating History

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Ann. Entomol. Soc. Am. 88(1): 100–103 (1995)

ABSTRACT Males of many insect species provide large ejaculates that females may use for somatic maintenance and egg production. Male cowpea weevils, *Callosobruchus maculatus* (F.), contribute large ejaculates in their first mating, averaging >5% of male body mass. Here, we quantify the influence of repeated matings on male ejaculate size in *C. maculatus* and find that multiply mated males produce much smaller ejaculates than once-mated males. Some of this effect, however, is compensated for if males increase the length of time between matings. Male age also has a large effect on ejaculate size; two-day-old males produce larger ejaculates than either young or old males.

KEY WORDS *Callosobruchus maculatus*, ejaculate size, multiple mating

MALES OF MANY insect species produce large ejaculates, and females that mate multiply may take advantage of male contributions for somatic maintenance and egg production (Thornhill 1976). Like other seed beetles, *Callosobruchus maculatus* (F.) males produce a large ejaculate (Huignard 1983, Boucher & Huignard 1987), averaging >5% of their body mass (Fox 1993a). Multiple mating by female *C. maculatus* has been demonstrated to have many benefits to females and their offspring (Wasserman & Asami 1985, Credland & Wright 1989, Fox 1993a, b), and these are likely the direct result of male nutritional contributions.

Experiments quantifying the benefits of multiple mating in *C. maculatus* have used newly emerged virgin males to control for variation in male contributions (Fox 1993a, b; Wasserman & Asami 1985); ejaculate and spermatophore size decrease with successive matings or increasing age in many insects (Outram 1971, Sims 1979, Svärd & Wiklund 1986, Royer & McNeil 1993). However, benefits demonstrated using only newly emerged virgin males may be unrepresentative of the benefits in a natural population because of natural variation in male mating history and age. In this article we quantify the influence of male age and mating history on ejaculate size in *C. maculatus*. We find that multiply mated males produce much smaller ejaculates than once-mated males, but that some of this effect can be compensated for if males increase the length of time between matings. Male age also has a large effect on male ejaculate size, with 2-d-old virgin males producing larger ejaculates than newly emerged males.

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Materials and Methods

Beetle populations were collected from stored azuki beans, *Vigna angularis*, near San Francisco, CA (details in Fox 1993c). The laboratory population was established with >1,000 eggs and maintained on azuki beans at 26–27°C, with 24 h of light.

The influence of repeated mating on ejaculate size was estimated in two groups of males. Males in one group (n = 30) were each mated four times, at ≈0.5-h intervals in the same afternoon. Males in another group (n = 14) were also mated four times, but on sequential days at ≈24-h intervals. The effect of male age on the size of his first ejaculate was estimated in males confined for 0, 2, 4, 6, and 8 d after emergence in a 30-mm petri dish without food or water (sample sizes in Table 1).

Ejaculate size was estimated by weighing males and females before and after mating. Each was weighed twice to 0.01-mg precision. If the two values differed by >0.04 mg, a third weighing was performed. Individual body weight was estimated as the average of these 2–3 values. Ejaculate size was calculated as (weight gain of female + weight loss of male)/2.

Virgin females <24-h post-adult emergence were used for all copulations. For the multiple mating experiment, all males were <24-h post-adult emergence before their first mating and were maintained without access to food or water during the experiment.

Table 1. Influence of male body size on the size of first ejaculate in *C. maculatus*

Age[a]	n	Regression	r^2
0[b]	44	$y = 0.11 + 0.02x$	0.04NS
0[c]	25	$y = 0.09 + 0.03x$	0.05NS
2	25	$y = -0.16 + 0.05x$	0.60***
4	25	$y = -0.06 \pm 0.10x$	0.37***
6	26	$y = -0.12 + 0.13x$	0.49***
8	10	$y = -0.08 + 0.11x$	0.09NS
Combined ages[d]	111	$y = 0.15 + 0.05x$	0.20***

NS, not significant; ***, $P < 0.001$; y, ejaculate size; x, body weight.

[a] Age 0 implies <24 h after emergence from the seed, age 2 is 48 h after age 0, and so on.

[b] Data from first mating of multiple mating experiment (Fig. 1).

[c] Data from male age experiment (Fig. 2).

[d] Data from male age experiment only.

Results

Male ejaculates averaged between 4.8 (mated each day; $n = 14$) and 5.2% (mated multiply in same day; $n = 30$) of their body weight during their first mating (Fig. 1). Males contributed significantly less of their body weight (Fig. 1A) and thus a significantly smaller ejaculate (Fig. 1B) in each subsequent mating (Wilcoxon signed rank test; each mating differed significantly from all others, $P < 0.05$). However, the decline in ejaculate size was significantly less in males allowed 24 h between copulations than in males mated repeatedly on the same day (Fig. 1; Mann–Whitney U-test, $P < 0.05$).

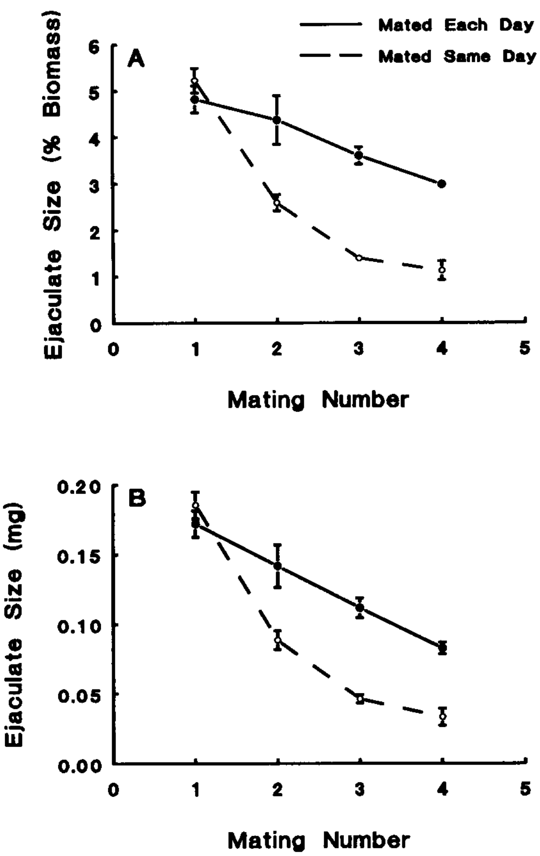

Fig. 1. The influence of multiple mating on ejaculate size of male *C. maculatus*. (A) The proportion of his body weight donated as ejaculate and (B) the absolute size of his first ejaculate. Males were mated at either 0.5- or 24-h intervals.

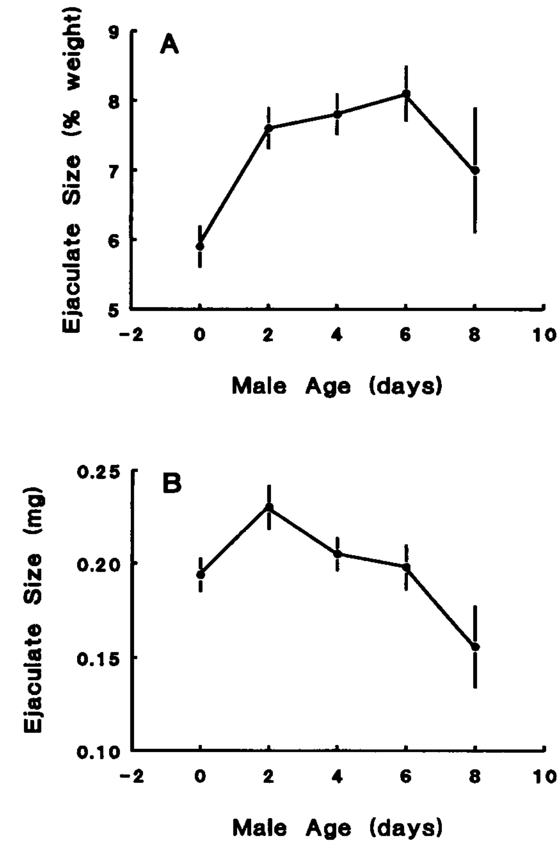

Fig. 2. The influence of male age on (A) the proportion of his body weight donated as ejaculate and (B) the absolute size of his first ejaculate, in *C. maculatus*.

Male age had a large effect on both absolute ejaculate size and the percentage of body weight included in the ejaculate (Kruskal–Wallis one-way analysis of variance, $P < 0.05$ and $P < 0.001$, respectively; sample sizes in Table 1). Two-day-old males produced larger ejaculates than either males mated immediately after emergence (Mann–Whitney U-test, $P < 0.05$) or males age 4–8 d (statistically different from age 8 d, but not ages 4 and 6; Mann–Whitney U-tests; Fig. 2B). However, because of weight loss as the males aged, their ejaculate size was an increasing percentage of their body weight until after 6 d of age (Fig. 2A).

The size of a male's first ejaculate was not significantly correlated with his body mass when ejaculate weight was estimated within 24 h of adult emergence (Table 1). However, for males of ages 2, 4, and 6 d, ejaculate weight was highly correlated with body weight (Table 1). Also, when males of all age classes were combined, ejaculate weight was positively correlated with male body weight (Table 1). However, when males of variable mating history were combined, there was no significant correlation between male body size and ejaculate size ($R^2 < 0.01$; $P > 0.05$).

Discussion

Our results indicated that multiply mated males of *C. maculatus* provide less ejaculate to females than do virgin males. This is important for the interpretation of experiments examining the effects of multiple mating on female and offspring life histories because females will frequently encounter ejaculate-depleted males in nature. Thus, laboratory experiments examining the benefits of multiple mating using virgin males may overestimate the benefits of multiply mating with nonvirgin males in nature.

As in *C. maculatus,* the time between matings affects subsequent ejaculate sizes in many insects (Boggs 1981). In *C. maculatus,* males allowed 24 h between matings produced larger ejaculates than males mated at 0.5-h intervals. However, males did not completely replenish their ejaculate. This suggests that replenishment occurs very slowly, that males lack the energy reserves to replenish their ejaculate fully, or both. A lack of energy reserves is a likely explanation because males are at least partially nutrient stressed in these experiments: they were not offered food or water. The effects of mating history and age on male ejaculate size will likely be different when males have access to food and water. However, nutrient stress is typical for *C. maculatus:* populations have been associated with stored legumes (dried seeds) for thousands of years (Mitchell 1983). Thus, their life cycle is well adapted for reproduction in a storage environment in which reproduction without access to food and water is typical for adults (Messina 1991).

That the size of a male's first ejaculate was correlated with his body weight in older males (ages 2–6 d), but not immediately after emergence, indicates that males do not emerge with their seminal vesicles and ejaculatory sac full. That 2-d-old males produced larger ejaculates than immediately after emergence confirms this. This is similar to the pattern observed in female *C. maculatus:* the number of mature eggs a female carries is not correlated with body weight immediately following emergence but is after 2 d (Wilson & Hill 1989). Similarly, the number of mature eggs a virgin female carries gradually increases during the first 2 d after emergence (Wilson & Hill 1989), as observed for male ejaculates.

In other insects, ejaculate or spermatophore size is generally correlated with male body mass (e.g., Svärd & Wiklund 1986, Goulson et al. 1993, Royer & McNeil 1993), although an absence of a correlation has also been reported (Svärd 1985). However, male age and mating history is often not controlled in these experiments. Our results indicated that, in a population of variable age males, body size is a useful indicator of a male's potential nutrient contribution if all males are virgin. However, if some males have previously mated, body size becomes an unreliable indicator of ejaculate size.

In these experiments, we have only examined ejaculate size as an indicator of paternal investment. However, ejaculate composition, rather than size, may be responsible for male effects on female life history (Marshall & McNeil 1989). For example, some males may donate small, lipid-rich ejaculates, whereas other males may donate larger, lipid-poor ejaculates. It is also unknown whether variation in ejaculate size is correlated with the number of sperm transferred, or whether some males are transferring a small ejaculate containing large quantities of sperm.

Acknowledgments

H. Dingle and J. Rosenheim provided laboratory space and equipment used during these experiments. L. McLennan provided helpful comments on a previous version of the manuscript. Financial support was provided by a National Institutes of Health training grant administered through the Genetics Graduate Group at the University of California at Berkeley.

References Cited

Boggs, C. L. 1981. Selection pressures affecting male nutrient investment at mating in Heliconiine butterflies. Evolution 35: 931–940.

Boucher, L. & J. Huignard. 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.

Credland, P. F. & A. W. Wright. 1989. Factors affecting females' fecundity in the cowpea seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). J. Stored Prod. Res. 25: 125–136.

Fox, C. W. 1993a. Multiple mating, lifetime fecundity and female mortality of the bruchid beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). Funct. Ecol. 7: 203–208.

1993b. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia (Berl.) 96: 139–146.

1993c. A quantitative genetic analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus.* Evolution 47: 166–175.

Goulson, D., M. C. Birch & T. D. Wyatt. 1993. Paternal investment in relation to size in the deathwatch beetle, *Xestobium rufovillosum* (Coleoptera: Anobiidae), and evidence for female selection of large mates. J. Insect Behav. 6: 539–547.

Huignard, J. 1983. Transfer and fate of male secretions deposited in the spermatophore of females of

Acanthoscelides obtectus Say (Coleoptera Bruchidae). J. Insect Physiol. 29: 55–63.

Marshall, L. D. & J. N. McNeil. 1989. Spermatophore mass as an estimate of male nutrient investment: a closer look in *Pseudoletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Funct. Ecol. 3: 605–612.

Messina, F. J. 1991. Life-history variation in a seed beetle: adult egg-laying vs. larval competitive ability. Oecologia (Berl.) 85: 447–455.

Mitchell, R. 1983. Effects of host-plant variability on the fitness of sedentary herbivorous insects, pp. 343–370. *In* R. F. Denno & M. S. McClure [eds.], Variable plants and herbivores in natural and managed systems. Academic, New York.

Outram, I. 1971. Aspects of mating in the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Entomol. 103: 1121–1128.

Royer, L. & J. N. McNeil. 1993. Male investment in the European corn borer, *Ostrinia nubialis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. Funct. Ecol. 7: 209–215.

Sims, S. R. 1979. Aspects of mating frequency and reproductive maturity in *Papilio zelicaon*. Am. Midl. Nat. 102: 36–50.

Svärd, L. 1985. Paternal investment in a monandrous butterfly, *Pararge aegeria*. Oikos 45: 66–70.

Svärd, L. & C. Wiklund. 1986. Different ejaculate delivery strategies in first versus subsequent matings in the swallowtail butterfly, *Papilio machaon* L. Behav. Ecol. Socioiol. 18: 325–330.

Thornhill, R. 1976. Sexual selection and paternal investment in insects. Am. Nat. 110: 153–163.

Wasserman, S. S. & T. Asami. 1985. The effect of maternal age upon fitness of progeny in the southern cowpea weevil, *Callosobruchus maculatus*. Oikos 45: 191–196.

Wilson, K. & L. Hill. 1989. Factors affecting egg maturation in the bean weevil *Callosobruchus maculatus*. Physiol. Entomol. 14: 115–126.

Received for publication 19 April 1994; accepted 1 September 1994.