Paternal Investment in the Seed Beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae): Variation Among Populations

UDO M. SAVALLI, MARY ELLEN CZESAK, AND CHARLES W. FOX

Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546–0091

Ann. Entomol. Soc. Am. 93(5): 1173–1178 (2000)

ABSTRACT Ejaculate size in seed beetles (Coleoptera: Bruchidae) is subject to both sexual and fecundity selection. We examined interpopulation variation and inheritance of ejaculate size in the seed beetle *Callosobruchus maculatus* (F.). There was significant variation among three populations in both body mass and the proportion of a male's body mass that was transferred to females during mating. The seed upon which beetles were raised had a small effect on male body size but not the size of their ejaculates. To investigate the inheritance of ejaculate size, we performed inter- and intrapopulation crosses with two of these populations. The progeny of interpopulation crosses were intermediate between the intrapopulation (parental) crosses, suggesting additive genetic autosomal inheritance. This result differs from an earlier study in which ejaculate size of a different population was maternally inherited. This study demonstrates that there is indeed genetic variation in ejaculate size, but that the loci exhibiting within-population variation may be different than the loci producing among-population variation.

KEY WORDS *Callosobruchus maculatus*, genetic variation, sex-linkage, ejaculate size, Bruchidae

IN MANY INSECTS, males produce large ejaculates containing nutrients that females use for reproduction or somatic maintenance (reviewed in Ridley 1988, Eberhard 1996, Vahed 1998). Females obtaining larger ejaculates can use these nutrients to lay more or larger eggs, and thus there is direct natural selection on males to produce large ejaculates. That is, fecundity selection is acting directly on male ejaculates (e.g., Savalli and Fox 1998a). Sexual selection can also favor the evolution of large ejaculates via sperm competition (Parker 1970, Smith 1984) or via female preference for males that provide larger ejaculates (e.g., Eberhard 1996, Savalli and Fox 1998a). For such paternal investment to evolve, however, there must be heritable variation in the size or nutrient content of male ejaculates or spermatophores. Although there has been considerable interest in the genetics of traits involved in sexual selection, including analyses of female preferences (reviewed in Ritchie 1992, Bakker and Pomiankowski 1995) and male secondary sexual traits (e.g., Cade 1984, Houde 1992, Hedrick 1994), few studies have examined the genetic traits relevant to paternal investment (see Sakaluk and Smith 1988, Savalli and Fox 1998b for exceptions).

In addition to the amount of genetic variation, the location of genes on chromosomes can also affect responses to selection. For example, recessive alleles are shielded from selection when heterozygous but are exposed to selection when hemizygous. Thus, selection acting on the heterogametic sex will lead to more rapid fixation of favorable recessive or partially recessive alleles if they are sex-linked rather than autosomal (Charlesworth et al. 1987). Sex-linkage can facilitate the evolution of sexual dimorphism and may be favored (via the translocation of loci to the sex chromosomes) if selection on a particular trait differs between males and females (Charlesworth et al. 1987, Rice 1984). However, there are few examples of sex-specific traits that are known to be sex-linked (e.g., Bennet-Clark and Ewing 1970, Grula and Taylor 1980, Kawanishi and Watanabe 1981, Thompson 1988, Houde 1992).

Ejaculate size has substantial fitness consequences for the seed beetle *Callosobruchus maculatus* (F.). Females obtaining multiple ejaculates or larger ejaculates (either from larger males or males that had not previously mated) have higher lifetime fecundity and lay larger eggs than females mating only once or obtaining smaller ejaculates (from small males or males that had previously mated) (Fox 1993a, 1993b; Savalli and Fox 1999a, 1999b). Furthermore, females obtaining larger ejaculates are less likely to remate than females obtaining small ejaculates, thereby reducing the risk of sperm competition for males that produce large ejaculates. Thus, both fecundity selection and sexual selection directly favor the evolution of large ejaculates in *C. maculatus.*

For ejaculate size to evolve, it must exhibit heritable variation. Previous experiments using traditional half-sib designs indicated that ejaculate size does exhibit heritable variation in one population of *C. maculatus.* This inheritance was primarily maternal, however (Savalli and Fox 1998b), indicating either sex-linkage (seed beetles have XY sex-determination) or a ma-

0013-8746/00/1173–1178$02.00/0

ternal effect (Mousseau and Dingle 1991, Mousseau and Fox 1998). Our experiments could not distinguish between these hypotheses, although we have argued that maternal effects are unlikely to account for the inheritance of ejaculate size (Savalli and Fox 1998b).

In this study we expand our investigation of the inheritance of paternal investment to comparisons among populations. Because sex-linked traits can respond more rapidly to selection than autosomal traits (Charlesworth et al. 1987), population differences in such traits is likely. Furthermore, there is reason to suspect population differences in the composition of ejaculates or how they are used by females. In a population collected from azuki beans in California, multiply mated females lived longer than singly mated females (Fox 1993a), but in a population collected from cowpeas in Niger, multiply mated females had shorter lives than singly mated females (Savalli and Fox 1999b). We therefore investigated differences among populations in ejaculate size, and tested for genetic differentiation among populations in male paternal investment in *C. maculatus.*

Materials and Methods

General Methods. *C. 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 seeds. 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. *C. maculatus* has nine pairs of autosomes and an XY sex determining mechanism in which males are the heterogametic sex (Smith and Brower 1974). We used beetles from three different populations. Beetles were collected from infested pods of cowpea, *Vigna unguiculata* (L.) Walp., at Niamey, Niger (NN population) and Ouagadougou, Burkina Faso (BF population) in 1989 and from infested pods of mung bean, *V. radiata* (L.) Wilczek, from Tirunelveli, southern India (SI population) in 1979 (details in Messina and Mitchell 1989, Messina 1993). All beetles were maintained at 26°C, 24 h light, on their original host in laboratory growth chambers at >1,500 adults per generation, before November 1997, when we began these experiments. Laboratory rearing conditions closely approximate the natural conditions (legume stores) of this species (Messina 1991), and all populations were reared on the host on which they were collected. Population differences are unlikely, therefore, to be artifacts of laboratory conditions. Voucher specimens will be submitted to the University of Kentucky Museum of Entomology.

Population Comparisons. To test for population differences in male ejaculate size, families were initiated with pairs of virgin males and females collected within 12 h of their adult emergence from haphazardly collected eggs laid in mass cultures. Because the populations were collected from and reared on different hosts, we needed to control for possible rearing host effects. Thus, half of the pairs from each population were provided with cowpeas and half with mung beans. We set up 20 pairs for each combination of host seed and population. Once these pairs mated, the females were placed on 10 seeds and allowed to lay eggs for 24 h. These eggs were reared to adults at densities of one larva per seed (females typically lay between 15 and 25 eggs under these circumstances; eggs in excess of one egg per seed were scraped off before hatching). Thus, each family was initiated from 10 eggs. We used only beetles reared from eggs laid during the first 24 h after mating to reduce the potential for maternal effects (because egg size varies with female age; Fox 1993a).

Virgin males and females emerging from these families were collected within 12 h of their adult emergence. We mated the first two or three males to emerge from each family to females of the same treatment. Males emerge with only partially filled seminal vesicles, with ejaculate size increasing over the first 2 d and then decreasing as males lose mass. As a result, ejaculate size is largest for males at ≈2 d old (Fox et al. 1995a). Thus, all virgin males were isolated in individual 35-mm petri dishes without seeds for 48 h before use in experiments, such that all males were of similar age, between 48 and 60 h old. We used females that were similar in age to the males, between 36 and 60 h old.

Ejaculate size was estimated by weighing males before and after mating. Before pairing, beetles were weighed twice to 0.01-mg precision on an electronic balance. If the two values differed by >0.03 mg, the male was weighed a third time. A male's mass was estimated as the average of these two to three values. After mating, beetles were reweighed as above. A male's ejaculate size was estimated as the amount of mass lost by the male during mating (mass of male before mating − mass of male after mating). In a previous experiment we demonstrated that female mass gain during mating is highly correlated with male mass loss ($r = 0.625$, $P < 0.001$; Savalli and Fox 1998b) and that females gain nearly as much (83%) mass as males lose. The difference between male mass loss and female mass gain is likely because of either spillage or expulsion of some ejaculate by females (Savalli and Fox 1998b), and for this reason we only measured male mass loss in this study. Mating lasts 5–10 min, and metabolic mass loss is probably negligible over such brief time periods. Each beetle was mated only once.

We also timed copulation duration during each mating. Copulation duration was defined as the period starting when a male stops drumming his antennae on the elytra of the female and stopping when the female begins kicking the male to remove him (Fox and Hickman 1994, Savalli and Fox 1998b).

We used analyses of variance (ANOVA) to test for population differences and rearing host effects on body size, male ejaculate size, and relative ejaculate size (proportion of a male's body mass transferred to the female). Family means were used in the analyses to control for non-independence among siblings.

Population Crosses. To test if the population differences obtained in the previous experiment are ma-

ternally inherited, we performed reciprocal crosses between two of the *C. maculatus* populations. We selected the two populations that had the largest difference in relative ejaculate size in the population comparisons, BF and SI, to maximize our ability to detect any meaningful patterns.

As in the previous experiment, beetles were collected within 12 h of their adult emergence from seeds collected from the mass cultures. These beetles were mated and their progeny were reared at low density (one egg per seed) before initiating the experiments. Because there were no large host effects on relative ejaculate size in the previous experiment, we reared all beetles on a common host, cowpea.

We set up four types of crosses: two sets of intrapopulation crosses (female BF × male BF and female SI × male SI) and two sets of interpopulation crosses (female SI × male BF and female BF × male SI). If inheritance is autosomal, we expect that the interpopulation crosses should resemble each other and be intermediate between the two intrapopulation crosses. If inheritance is maternal (either because of sex-linkage or maternal effects), we expect that the offspring of the interpopulation crosses will most closely resemble their respective maternal population.

As in the previous experiment, progeny from the crosses were reared at one larva per seed from eggs laid on 10–12 seeds during the first 12 h after mating. We set up 43–45 families per cross, but because some families did not produce any male progeny, we had a total of 167 families (38–45 families per cross).

Virgin male and female offspring were collected within 12 h of their adult emergence from the seeds. As before, virgin beetles were isolated from each other and allowed to mature for 48 h before mating. We mated the first four males to emerge from each family (occasionally fewer, if less than four males emerged from a family) to females of the same cross whenever possible (in 14 cases, we had no females of the appropriate cross available, so we substituted females from other crosses; this does not pose any problems because females do not influence ejaculate size [Savalli and Fox 1998b]). As before, ejaculate size was estimated by weighing males two to three times to 0.01-mg precision before and after mating. Because copulation duration differed only slightly between these two lines in the previous experiment, we did not consider copulation duration in our crosses.

Results

Population Comparisons. There was significant variation in male body size among the three populations (Table 1) and between the two rearing hosts: the SI population was largest, whereas the BF and NN populations were very similar to each other, and males from the SI population raised on cowpea were larger than males reared on mung bean (mean ± SE for body mass in milligrams; cowpea, SI 5.0 ± 0.8, NN 3.8 ± 0.8, BF 3.8 ± 0.9; mung, SI 4.6 ± 1.0, NN 3.8 ± 0.5, BF 3.7 ± 0.8). There was also significant variation in relative ejaculate size (the proportion of the male's body mass transferred to the female) among the populations, but the pattern was reversed: SI males had the smallest relative ejaculate size (Fig. 1). As a consequence of these patterns, the variation in body size and variation in relative ejaculate size canceled each other, resulting in no detectable among-population variation in absolute ejaculate size. In two of the populations, body size was positively correlated with absolute ejaculate size (BF, $r = 0.46$, $n = 85$, $P < 0.001$; SI, $r = 0.41$, $n = 77$, $P < 0.001$) but not with relative ejaculate size (BF, $r = -0.096$, $n = 85$, $P = 0.38$; SI, $r = -0.142$, $n = 77$, $P = 0.22$). In the NN population, however, there was a significant negative correlation between body size and relative ejaculate size (larger males donated a smaller proportion of their body mass; $r = -0.287$, $n = 76$, $P = 0.012$) but no correlation between body size and absolute ejaculate size ($r = 0.036$, $n = 76$, $P = 0.76$).

Table 1. ANOVA testing for effects of source population and rearing host on male body size and ejaculate size in the seed beetle *C. maculatus* ($n = 119$ families)

Factor	df	Male mass		Absolute ejaculate size		Relative ejaculate size	
		F	*P*	*F*	*P*	*F*	*P*
Population	2, 113	104.5	<0.001	2.10	0.13	15.7	<0.001
Host	1, 113	8.08	0.005	3.68	0.057	0.45	0.51
Population*Host	2, 113	4.07	0.020	0.43	0.65	0.062	0.94

There was a significant effect of rearing host (cowpea or mung bean) and a significant host × population interaction, on male body size, but not on either measure of ejaculate size (Table 1). The duration of copulation also varied among populations (longest for NN and shortest for SI; $P < 0.001$) and rearing hosts (longest for cowpea reared beetles; $P < 0.01$), but there was no host × population interaction and no relationship between a male's ejaculate size and copulation duration ($P > 0.26$ for both absolute and relative ejaculate size, mean copulation duration ± SE in minutes; cowpea, NN 8.21 ± 0.61, BF 6.42 ± 0.34, SI 5.90 ± 0.34; mung, NN 7.50 ± 0.55, BF 5.81 ± 0.29, SI 4.72 ± 0.25).

Population Crosses. Overall, there were statistically significant effects of both the paternal population and

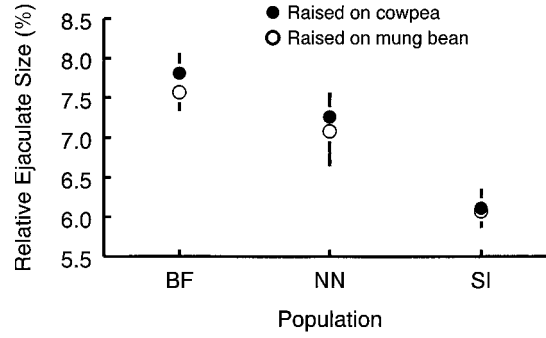

Fig. 1. Mean ± SE relative ejaculate size (percentage of body mass) for males of three populations of *Callosobruchus maculatus* that were reared on two hosts.

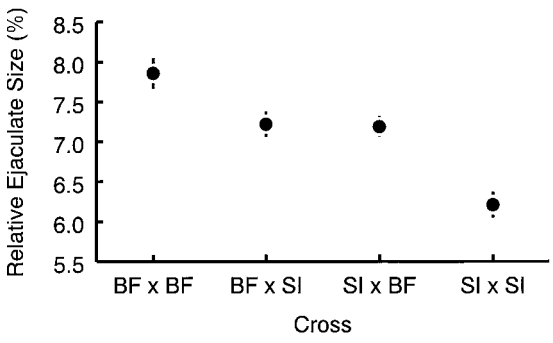

Fig. 2. Mean ± SE relative ejaculate (percentage of body mass) sizes of males from population crosses. Crosses are depicted as female population × male population.

the maternal population on a male's relative ejaculate size (maternal population: $F = 26.8$; df = 1, 166; $P < 0.001$; paternal population, $F = 25.0$; df = 1, 166; $P < 0.001$). The interaction between maternal and paternal population was not statistically significant ($F = 1.10$; df = 1, 1; $P = 0.30$). There was, however, no evidence of maternal effects or sex linkage, and offspring of the two interpopulation crosses did not differ from each other and were intermediate between the two parental crosses (Fig. 2).

Discussion

Our results demonstrate that there are population differences in the proportion of biomass that males transfer to females during mating. We expected to find variation in ejaculate size because populations differ in body size and because ejaculate size and body size are positively correlated (Savalli and Fox 1998a, 1998b). We were thus surprised not to find any variation among populations in absolute ejaculate size. The variation in male size and in the proportion of their body mass contributed to females in their first ejaculate appear to have canceled each other out, resulting in no detectable differences in absolute ejaculate size. Different populations have thus evolved different degrees of relative ejaculate size. The functional significance of this variation is not known. Large ejaculates are favored by sexual selection (Savalli and Fox 1999a) and by fecundity selection (Savalli and Fox 1999b; see also Savalli and Fox 1998a for another seed beetle). Previous work has suggested that there may be differences in the composition or use of ejaculates in *C. maculatus*—multiple mating by females enhanced their survival in one population but decreased it in another (Fox 1993a, Savalli and Fox 1999b)—but beyond this, interpopulation variation in selection on paternal investment has not been investigated. We do not know if there are interpopulation differences in ejaculate composition. Relatively few studies have investigated geographic variation in sexually selected traits (but see Ryan et al. 1992, Houde 1993, Hill 1994, Savalli 1995 for some exceptions), and we know of no other study to examine geographic variation in paternal investment.

Crosses among two of the populations did not support our earlier finding—based on a within-population design using the NN population (Savalli and Fox 1998b)—that variation in relative ejaculate size is maternally inherited. Instead, the two interpopulation crosses were intermediate between the parental crosses, suggesting additive autosomal inheritance. Alternatively, the crosses would be intermediate in relative ejaculate size if (1) there is no genetic difference between populations in absolute ejaculate size and (2) ejaculate size segregates independently of body size. In this case, smaller beetles will produce on average the same size ejaculates as larger beetles but these ejaculates will be a larger proportion of their body mass. That this is not the case is indicated by the fact that absolute ejaculate size is positively correlated with body mass, and relative ejaculate size does not correlate with body mass in the two populations used for the crosses. Thus, body size differences between populations are not likely to explain the difference between populations in relative ejaculate size. It is more likely that the difference in relative ejaculate size represents an autosomally inherited difference between populations.

There are three possible explanations for why we obtained evidence for autosomal inheritance in this study while we observed sex-linked variation in our within-population study. First, there may be detectable maternal effects operating within the NN population but not among other populations. We have argued elsewhere (Savalli and Fox 1998b) that maternal effects were unlikely to account for the pattern of maternal inheritance observed within the NN population. The fact that we did not detect any evidence of maternal effects among other populations (where differences between females should be even greater) lends further credence to this view. Second, the mode of inheritance of variation in ejaculate size may differ among populations, being primarily autosomal in the BF and SI populations and sex linked in the NN population. Lastly, the populations may be fixed for different alleles at autosomal loci, so that these loci produce among-population variation similar to that observed in this study. However, in these same populations ejaculate size may be influenced by one or more sex-linked loci that vary within populations but not among populations.

There has been substantial interest in the evolution of male parental investment and sperm competition (e.g., Clutton-Brock 1991; Birkhead and Møller 1992). Male ejaculate size can influence female fecundity and egg or offspring size and quality because nutrients within the ejaculate are used by females during oogenesis and thus influence a male's fitness via nongenetic contributions to his offspring (e.g., Thornhill and Alcock 1983; Ridley 1988; Fox et al. 1995b; Savalli and Fox 1998a, 1999b). In *C. maculatus*, females that receive multiple ejaculates lay more and larger eggs and in some cases may live longer than once-mated females (Fox 1993a, 1993b). Ejaculate size may also affect the outcome of sperm competition by reducing the likelihood that a female will remate. Female *C.*

maculatus mating with previously mated males and thus receiving small ejaculates remate more readily than females mating with virgins (Savalli and Fox 1999b). Sperm competition by swamping another male's sperm (Parker 1970; Smith 1984) may also be important in *C. maculatus* (Eady 1995) and could lead to the evolution of large ejaculates if sperm number affects ejaculate size. Producing large ejaculates may also incur costs such as reduced lifespan or subsequent sperm depletion that results in few future matings. Despite such potential costs and benefits to producing large ejaculates, few studies have demonstrated that there is heritable variation in ejaculate size. Our study demonstrates that there is genetic variation in male investment via ejaculates that is independent of body size. Although the variation in ejaculate size in one population appears to be sex-linked, the variation among populations exhibits an autosomal pattern of inheritance, suggesting that the loci exhibiting within-population variation are different from the loci that produce the among-population variation.

Acknowledgments

F. J. Messina kindly provided the beetle populations. We thank Jason Cope, Paul Spinelli, and two anonymous reviewers for helpful comments on the manuscript. This is publication number 99-08-159 of the University of Kentucky Agricultural Experiment Station.

References Cited

Bakker, T.C.M., and A. Pomiankowski. 1995. The genetic basis of female mate preferences. J. Evol. Biol. 8: 129–171.

Bennet-Clark, H. C., and A. W. Ewing. 1970. The love song of the fruit fly. Sci. Am. 223(7): 84–92.

Birkhead, T. R., and A. P. Møller. 1992. Sperm competition in birds. Academic, New York.

Cade, W. H. 1984. Genetic variation underlying sexual behavior and reproduction. Am. Zool. 24: 355–366.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130: 113–146.

Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton University Press, Princeton, NJ.

Eady, P. 1995. Why do male *Callosobruchus maculatus* beetles inseminate so many sperm? Behav. Ecol. Sociobiol 36: 25–32.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ.

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

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

Fox, C. W., and D. L. Hickman. 1994. Influence of oviposition substrate on female receptivity to multiple mating in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Ann. Entomol. Soc. Am. 87: 395–398.

Fox, C. W., D. L. Hickman, E. L. Raleigh, and T. A. Mousseau. 1995a. Paternal investment in a seed beetle (Coleoptera: Bruchidae): influence of male size, age, and mating history. Ann. Entomol. Soc. Am. 88: 101–103.

Fox, C. W., L. A. McLennan, and T. A. Mousseau. 1995b. Male body size affects female lifetime reproductive success in a seed beetle. Anim. Behav. 50: 281–284.

Grula, J. W., and O. R. Taylor, Jr. 1980. The effect of X-chromosome inheritance on mate-selection behavior in the sulfur butterflies, *Colias eurytheme* and *C. philodice.* Evolution 34: 688–695.

Hedrick, A. V. 1994. The heritability of mate-attractive traits: a case study on field crickets, pp. 228–250. *In* C.R.B. Boake [ed.], Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago, IL.

Hill, G. E. 1994. Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. Behav. Ecol. 5: 64–73.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). Heredity 69: 229–235.

Houde, A. E. 1993. Evolution by sexual selection: what can population comparisons tell us? Am. Nat. 141: 796–803.

Kawanishi, M., and T. K. Watanabe. 1981. Genes affecting courtship song and mating preference in *Drosophila melanogaster, Drosophila simulans,* and their hybrids. Evolution 35: 1128–1133.

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

Messina, F. J. 1993. Heritability and 'evolvability' of fitness components in *Callosobruchus maculatus.* Heredity 71: 623–629.

Messina, F. J., and R. Mitchell. 1989. Intraspecific variation in the egg-spacing behavior of the seed beetle *Callosobruchus maculatus.* J. Insect Behav. 2: 727–742.

Mousseau, T. A., and H. Dingle. 1991. Maternal effects in insect life histories. Annu. Rev. Entomol. 36: 511–534.

Mousseau, T. A., and C. W. Fox [eds.]. 1998. Maternal effects as adaptations. Oxford University Press, New York.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences. Biol. Rev. 45: 525–567.

Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38: 735–742.

Ridley, M. 1988. Mating frequency and fecundity in insects. Biol. Rev. 63: 509–549.

Ritchie, M. G. 1992. Setbacks in the search for mate-preference genes. Trends Ecol. Evol. 7: 328–329.

Ryan, M. J., S. A. Perrill, and W. Wilczynski. 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans.* Am. Nat. 139: 1370–1383.

Sakaluk, S. K., and R. L. Smith. 1988. Inheritance of male parental investment in an insect. Am. Nat. 132: 594–601.

Savalli, U. M. 1995. Does rainfall constrain the evolution of tail length in widowbirds? Ethol. Ecol. Evol 7: 379–385.

Savalli, U. M., and C. W. Fox. 1998a. Sexual selection and the fitness consequences of male body size in the seed beetle *Stator limbatus.* Anim. Behav. 55: 473–483.

Savalli, U. M., and C. W. Fox. 1998b. Genetic variation in paternal investment in a seed beetle. Anim. Behav. 56: 953–961.

Savalli, U. M., and C. W. Fox. 1999a. The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus.* Ethol. Ecol. Evol. 10: 49–60.

Savalli, U. M., and C. W. Fox. 1999b. The effect of male mating history on paternal investment, fecundity, and

female remating in the seed beetle *Callosobruchus maculatus.* Funct. Ecol. 13: 169–177.

Smith, R. L. [ed.]. 1984. Sperm competition and the evolution of animal mating systems. Academic, New York.

Smith, S. G., and J. H. Brower. 1974. Chromosome numbers of stored-product Coleoptera. J. Kans. Entomol. Soc. 47: 317–328.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Thompson, J. N. 1988. Evolutionary genetics of oviposition preference in swallowtail butterflies. Evolution 42: 1223–1234.

Vahed, K. 1998. The function of nuptial feeding in insects: a review of empirical studies. Biol. Rev. 73: 43–78.

Received for publication 17 December 1999; accepted 27 April 2000.