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Genetic variation in paternal investment in a seed beetle

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ABSTRACT

Males of many species invest resources in their offspring. For paternal investment to evolve, it must exhibit heritable variation. Using a standard half-sibling quantitative genetic design, we investigated whether genetic variation in male ejaculate size, a trait that affects female fecundity and copulation duration, are present in the seed beetle *Callosobruchus maculatus*. Ejaculate size was estimated as the amount of weight lost by males during mating. Dams, but not sires, had significant effects on their sons' absolute ejaculate size (both replicates) and relative ejaculate size (proportion of body weight; one replicate only), explaining 21–25% of the variance in absolute ejaculate size and 8–16% of the variance in relative ejaculate size. These results suggest either a large maternal effect on ejaculate size or sex-linkage of loci that affect the variation in ejaculate size. The proportion of phenotypic variance explained by sex-linkage (assuming no maternal effects) was 42 and 49% (ejaculate size) and 17 and 31% (relative ejaculate size) in the two replicates. These results indicate that male paternal investment can respond to selection, and that it may be able to do so especially rapidly because sex-linked traits have the potential to evolve much more quickly than autosomal traits. There were only weak negative correlations between ejaculate size and mating duration, contrary to what we predicted. There was additive genetic variation in female copulation duration, but not in male copulation duration, suggesting that copulation duration is under female control.

Male parental investment in offspring is often an important component of male reproductive tactics (Clutton-Brock 1991). In insects and other organisms, males can provide considerable nutrients via ejaculates or spermatophores (Thornhill 1976; Thornhill & Alcock 1983) and females obtaining more or larger spermatophores lay more or larger eggs (e.g. Thornhill 1976; Thornhill & Alcock 1983; Ridley 1988; Fox et al. 1995b; Eberhard 1996). Fecundity selection may thus favour males that can produce large ejaculates (e.g. Savalli & Fox 1998). If nutrients from ejaculates or spermatophores can be used by females for somatic maintenance and egg production, it should benefit females to choose males that can provide large ejaculates. Thus, sexual selection via female choice can favour the evolution of large ejaculates. On the other hand, large paternal contributions may reduce male survivorship (Partridge & Andrews 1985) or reduce future mating opportunities (Dewsbury 1982; Parker 1984; Birkhead & Fletcher 1992; Pitnick 1993; Pitnick & Markow 1994), favouring the evolution of reduced ejaculate size.

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For paternal investment to evolve 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 & Pomiankowski 1995), and male secondary sexual traits (e.g. Cade 1984; Houde 1992; Hedrick 1994), few studies have examined the genetics of traits relevant to paternal investment in offspring. A notable exception is the demonstration that, in the cricket *Gryllodes supplicans*, the relative size of the spermatophylax (the portion of the spermatophore that is eaten by females and used for egg production) is heritable (h^2=0.47; Sakaluk & Smith 1988).

In addition to the amount of genetic variation, the location of genes on chromosomes can 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 favourable recessive or partially recessive alleles if they are sex-linked rather than autosomal (Charlesworth et al. 1987). Sex-linkage can even facilitate the evolution of sexual dimorphism, and may be favoured (via the translocation of loci to the sex chromosomes) if selection on a particular

trait differs between males and females (Rice 1984; Charlesworth et al. 1987). Other than loci affecting the viability of interspecific hybrids, there are relatively few examples of sex-specific traits that are known to be sex-linked (e.g. Bennet-Clark & Ewing 1970; Grula & Taylor 1980; Kawanishi & Watanabe 1981; Thompson 1988; Houde 1992).

In seed beetles (Coleoptera: Bruchidae), radiolabelled nutrients in male ejaculates are incorporated into both somatic and reproductive tissues of females (Boucher & Huignard 1987; Huignard 1983), and these nutrients are used by females during egg production. Females that receive multiple ejaculates live longer, lay more eggs and lay larger eggs than once-mated females (Fox 1993a, b; Fox et al. 1995a b). Males of some species produce large ejaculates that contain up to seven times the sperm that can be retained in the female's spermatheca, suggesting that sperm competition may also play a role in the evolution of large ejaculates (Eady 1995).

In this study we examine genetic variation in male paternal investment (ejaculate size) in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). In particular, we demonstrate that at least part of this genetic variation is probably sex-linked, which should favour especially rapid responses to selection. We also examine genetic variation in mating duration and demonstrate that female mating duration is genetically variable, but that male mating duration is not (indicating that females control mating duration in this insect).

METHODS

Callosobruchus maculatus is a cosmopolitan pest of stored legumes (Fabaceae). Females cement their eggs to the surface of host seeds (Messina 1991) and larvae burrow into the 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. This beetle's short generation time (about 4 weeks) and ease of laboratory rearing make it an excellent subject for genetic and life history studies. *Callosobruchus maculatus* has nine pairs of autosomes and, like most insects, an XY sex-determining mechanism in which males are the heterogametic sex (Smith & Brower 1974). All beetles used in these experiments were collected from infested pods of cowpea (*Vigna unguiculata*) in Niamey, Niger, at the University of Niamey Experiment Station, in November 1989, and maintained in laboratory growth chambers at more than 1500 adults per generation, prior to this experiment.

A traditional half-sibling design was used to estimate genetic effects on male ejaculate size and copulation duration (Falconer 1989). We performed two replicates of the experiment. Twenty-one sires (replicate 1) and 10 sires (replicate 2) were each mated to three or four different dams, creating 106 full-sibling families (nine females did not mate or produce eggs). After excluding those families with less than two male offspring, we obtained data from 95 families.

Half-sibling families were initiated with virgin males and females collected within 12 h of their adult emergence from haphazardly collected eggs laid in a mass culture. Beetles were reared at one larva per seed. To reduce the potential for maternal effects (Mousseau & Dingle 1991), we only used beetles raised from eggs laid during the first 24 h following mating (since egg size varies with female age; Fox 1993a). Because males emerge with only partially filled seminal vesicles, ejaculate size is largest for males approximately 2 days old (Fox et al. 1995a). Thus, all virgin males were isolated from each other in individual 35-mm petri dishes without seeds and allowed to mature for 48 h before use in experiments, such that all male parents were of similar age, between 48 and 60 h old. Similarly, we used females that were similar in age to the males, between 36 and 60 h old. To estimate the heritability of ejaculate size using a parent–offspring regression (for comparison with estimates from the half-sibling experiment), we measured ejaculate size and copulation during (see below) for each male in replicate 1 during his copulation with his first dam. We only recorded ejaculate size and copulation duration for the first dam to mate with a male because ejaculate size declines and copulation duration increases with subsequent matings (Fox et al. 1995a; Savalli & Fox, in press).

Mated females were placed on 10–12 seeds for 24 h and allowed to lay eggs. These eggs were reared to adulthood at densities of one beetle per seed (females typically lay between 15 and 25 eggs under these circumstances; excess eggs were haphazardly chosen and scraped off prior to hatching). Thus, we reared 10–12 progeny per family. As in the previous generation, virgin males and females were collected within 12 h of their adult emergence, isolated from each other in individual 35-mm petri dishes without seeds, and allowed to mature for 48 h before being mated to determine ejaculate size and copulation duration. We mated the first four offspring to each sex to emerge from each family (occasionally fewer, if fewer than four beetles of a sex emerged from one family, or more, if additional beetles of a sex were needed), but excluded those families with fewer than two males emerging, for a total of 712 offspring (446 for replicate 1; 266 for replicate 2) or an average of 3.7 offspring of each sex per family.

Ejaculate size was estimated by weighing both males and females 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 more than 0.04 mg, a third weighing was performed. A beetle's weight was estimated as the average of these two or three values. Following mating, beetles were reweighed as above. A male's ejaculate size was estimated as the amount of weight lost by the male during mating (weight of male before mating − weight of male after mating). Male weight loss was compared to female weight gain to quantify the amount of ejaculate that was lost during mating.

Once paired, the beetles were observed continuously until they finished mating. Courting males approach females from behind and antennate their backs while attempting to mount. While clasping the female with his pro- and mesothoracic legs, the male antennates and palpates the sides of the female and extends his aedeagus

under the female's abdomen, attempting to contact her genitalia (Rup 1986; Fox & Hickman 1994). Once males successfully insert their aedeagus, they cease waving their antennae and lean back, remaining motionless. We scored this coincident shift in posture and behaviour as the initiation of mating. Once mating is completed, females begin kicking at the male with their hind legs in an attempt to remove the male (separation appears difficult and can take several minutes; males do not play an active role in the separation attempt). We scored a mating as completed when the female began kicking. Mating duration was calculated as the time, to the nearest 10 s, between the initiation and completion of mating.

We examined genetic and maternal influences on ejaculate size using the restricted maximum likelihood variance component estimation procedure of SAS (SAS VARCOMP method=REML; SAS Institute 1985). We estimated the proportion of phenotypic variance (V_P) explained by additive genetic effects (V_A) as $4 \times V_S$ (the between-sire variance component). We calculated standard errors for the proportion of V_P explained by V_A (i.e. the heritability, h^2) following Becker (1984). We calculated the maternal effects variance (V_M) assuming that the dominance variance and higher-order interactions (e.g. V_{AA}, V_{AAA}) were 0. See Fox (1994, 1997) for other examples of these procedures.

RESULTS

Male weight loss during mating was highly correlated with female weight gain. However, males tended to lose more weight than females gained (Fig. 1), suggesting that there is either some spillage or that females expel some of the ejaculate after mating (a possible form of cryptic female choice; Eberhard 1996). Mean ($\pm$ SE) ejaculate size in *C. maculatus* was 0.31 ($\pm$ 0.01) mg (7.9 $\pm$ 0.12% of a male's body mass) for replicate 1 and 0.28 ($\pm$ 0.01) mg (7.2 $\pm$ 0.13% of body mass) for replicate 2. Male ejaculate size was positively correlated with male body size prior to mating; that is, larger males produced larger ejaculates (linear regression analyses; replicate 1: R^2=0.096, P<0.001; replicate 2: R^2=0.241, P<0.001). Similar results were obtained when the family means were compared (Fig. 2); if ejaculate size is maternally inherited and maternal or common environmental effects on ejaculate size are small, then the family mean correlations approximate the genetic correlations between ejaculate size and body size. There were only weak negative correlations between ejaculate size and mating duration (linear regression analyses; replicate 1: R^2=0.015, P=0.039; replicate 2: R^2=0.035, P=0.020), suggesting that mating duration is at best a weak predictor of male investment.

In a nested analysis of variance, with dams nested within sires, dams but not sires had significant effects on their sons' ejaculate size for both replicates (Table 1). A similar pattern, significant for one replicate, was obtained for relative ejaculate size (proportion of a male's body mass donated to the female). Using a genetic model that assumes no sex-linkage, there was no evidence of any additive genetic variation in ejaculate size; that is, the between-sire variance component was nonsignificant and

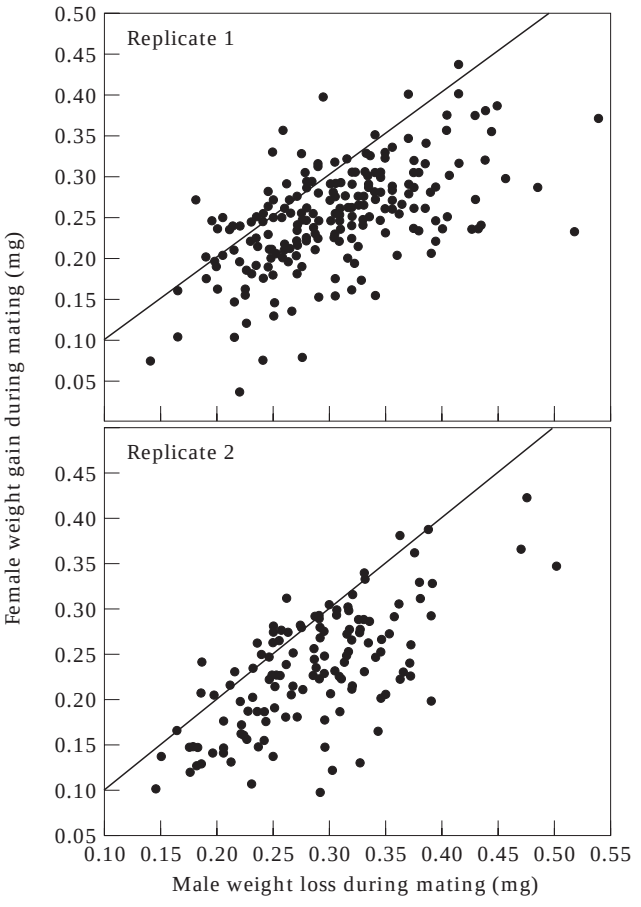

Figure 1. The relationship between female weight gain and male weight loss as a result of ejaculate transfer during mating in the seed beetle *Callosobruchus maculatus.* Note that most observations fall below the lines of equality.

the estimated V_A using SAS VARCOMP (SAS Institute 1985) was 0 (i.e. h^2=0) for both replicates. However, the between-dam variance component was highly significant in both replicates, explaining 21 and 25% of the variance in ejaculate size in replicates 1 and 2, respectively (Table 1). Similarly, there was no evidence of additive genetic variation in relative ejaculate size (again assuming no sex-linkage), although dam effects explained 8 and 16% of the variance in replicates 1 and 2. These results are suggestive of either a large maternal effect on ejaculate size or sex-linkage of loci that affect the variation in ejaculate size. Assuming no maternal effects, the proportion of phenotypic variance explained by sex-linkage (see Becker 1984) was 42 and 49% (ejaculate size) and 17 and 31% (relative ejaculate size) for replicates 1 and 2 (Table 2).

We performed parent–offspring analyses of ejaculate size of fathers and sons for replicate 1 (we did not obtain ejaculate size for fathers in replicate 2). The heritabilities obtained from these analyses were small and did not differ statistically from 0 (h^2: ejaculate size: 0.109 $\pm$ 0.278; relative ejaculate size: 0.054 $\pm$ 0.265; NS for both), a result consistent with the half-sibling analyses. Because ejaculate size is a character expressed only in males, it is not

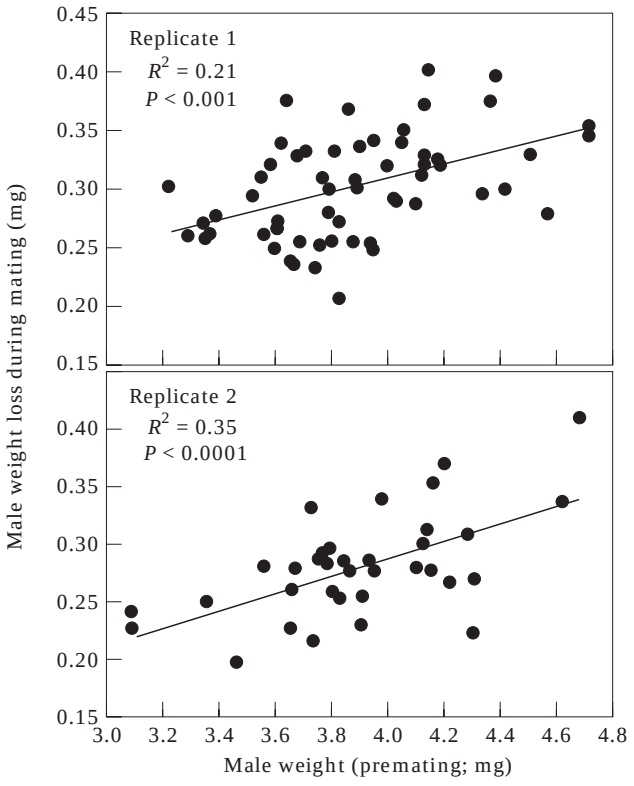

Figure 2. The relationship between the full-sibling family averages of male ejaculate size, measured as male weight loss during mating, and male size (prior to mating) in the seed beetle *Callosobruchus maculatus*. If ejaculate size is maternally inherited and maternal effects or common environmental effects on ejaculate size are small, then these correlations approximate the genetic correlations between ejaculate size and body size.

possible to detect genetic variation that is inherited via the X-chromosome, or to examine maternal effects, using parent–offspring regression.

Because both males and females can potentially influence copulation duration, we investigated genetic variation in male and female mating duration separately. Copulations lasted an average of 5.3 (± 0.12) min. Only the female's sire affected copulation duration (Table 3), suggesting that copulation duration is under female control (which accords with behavioural observations) and that there is additive genetic variation in female copulation duration (h^2=0.25 and 0.35 for replicates 1 and 2, respectively), but not in male copulation duration (Table 4). There were no correlations between the female full-sibling family means for mating duration and male full-sibling family means for ejaculate size (linear regression analyses; replicate 1: R^2=0.0002, P=0.92; replicate 2: R^2=0.032, P=0.33), indicating that these traits are not genetically correlated. This result is consistent with the possibility that ejaculate size is sex-linked while mating duration is autosomal.

DISCUSSION

Our results demonstrate that mothers, but not fathers, affect a male offspring's ejaculate size. This indicates that either (1) maternal effects, which are nongenetic factors that are passed from parent to offspring (Mousseau & Dingle 1991), influence the characteristics of the offspring or (2) some or all of the loci influencing ejaculate size are on the X-chromosome (males inherit their X-chromosome exclusively from their mother).

That ejaculate size is influenced by maternal effects seems unlikely because it is hard to imagine a mechanism by which ejaculate size could be inherited nongenetically from an individual's mother. Studies that have examined the types of characters influenced by maternal effects suggest that, although maternal effects can have large influences on progeny phenotype early in ontogeny, they rarely affect progeny later in ontogeny (Roach & Wulff 1987; Mousseau & Dingle 1991; Fox 1997; Mousseau & Fox 1998). For example, in the seed beetle *Stator limbatus*, very dramatic differences in body size (up to an order of magnitude) produced by variable rearing conditions do not lead to detectable phenotypic effects in adult offspring (Fox 1997). Similarly, maternal effects on *C. maculatus* body size have not been detectable. For example, egg size is associated with maternal age but has no effect on progeny body size (Fox 1993a, 1994). In the present experiment, all beetles were reared under identical conditions of low density (one beetle per seed) from eggs laid within 24 h of mating, so there were no dramatic environmental differences among the families that could result in the observed pattern. We found no evidence of maternal effects on the body size of *C. maculatus* when females and progeny were reared at low density (Fox & Savalli 1998).

The alternative explanation, that at least some of the loci influencing ejaculate size are on the X-chromsome, is more plausible. Ejaculate size is expressed only in males, which are heterogametic, and loci on the X-chromosome are always in the hemizygous state in males and thus always expressed. Consequently, sex-linked traits have the potential to evolve much more quickly than autosomal traits if they are at least partially recessive (Charlesworth et al. 1987). However, although sex-linkage is a more plausible explanation than maternal effects, our data do not allow us to distinguish between these hypotheses. A three-generation experiment, testing the effect of grandparents on grandoffspring, would enable environmentally based maternal effects to be distinguished from sex-linkage or genetically based maternal effects, but we know of no way, given the limited knowledge of the genetics of this species, to distinguish sex-linkage from genetically based maternal effects.

We have made a number of assumptions during our parameter estimation and interpretation. We have assumed that there were no dominance or epistatic effects and that variation in ejaculate size is controlled by multiple loci. These assumptions are common to the interpretation of half-sibling designs because such designs provide no means of distinguishing these effects. In fact, it is difficult to measure dominance and epistatic effects and to determine how many loci govern quantitative traits in any organism (Barker 1979; Roff 1997). For morphological traits, the assumption of no dominance seems generally to be warranted (Crnokrak & Roff 1995;

Table 1. Nested analyses of variance and observational and genetic variance components (assuming autosomal inheritance) for ejaculate size of male *Callosobruchus maculatus*

	Replicate 1						Replicate 2					
	ANOVA				Variance components		ANOVA				Variance components	
Character/Source	*df*	*MS* ($\times 10^{-3}$)	*F*	*P*	Observational ($\times 10^{-3}$)	Genetic ($\times 10^{-3}$)	*df*	*MS* ($\times 10^{-3}$)	*F*	*P*	Observational ($\times 10^{-3}$)	Genetic ($\times 10^{-3}$)
Ejaculate size												
Size	20	5.53	0.65	0.852	$V_S=0$	$V_A=0$	9	7.23	1.02	0.448	$V_S=0.04$	$V_A=0.14=3.3\pm7.7\%$
Dam (sire)	47	8.38	2.28	<0.001	$V_D=1.01$	$V_M=1.01=21.0\%$	28	7.12	2.22	0.002	$V_D=1.10$	$V_M=1.07=24.6\%$
Error	155	3.68			$V_E=3.78$	$V_e=3.78=79.0\%$	96	3.21			$V_E=3.21$	$V_e=3.14=72.2\%$
Ejaculate size (proportional body size)												
Sire	20	0.38	1.09	0.390	$V_S=0$	$V_A=0$	9	0.20	0.65	0.745	$V_S=0$	$V_A=0$
Dam (sire)	47	0.35	1.30	0.120	$V_D=0.03$	$V_M=0.03=8.5\%$	28	0.30	1.69	0.031	$V_D=0.03$	$V_M=0.03=15.6\%$
Error	155	0.27			$V_E=0.27$	$V_e=2.73=91.5\%$	96	0.17			$V_E=0.18$	$V_e=0.18=84.4\%$

Separate analyses for each replicate are presented to emphasize that the large dam effects are replicable. Type III sums of squares were calculated using SAS GLM (SAS Institute 1985). Variance components were estimated using the restricted maximum likelihood method of SAS VARCOMP. The maternal effects variances (V_M) were calculated assuming no sex-linkage and that dominance variance and higher-order interactions (e.g. V_{AA}, V_{AAA})=0. Standard errors for the percentage V_P (phenotypic variance) explained by additive genetic variance (V_A) were calculated following Becker (1984). V_S=between-sire variance, V_D=between-dam variance, V_E=error variance in ANOVA, V_e=environmental variance.

Table 2. Sex-linkage variance components and the proportion of total phenotypic variance explained by sex-linkage variance (assuming no maternal effects) for ejaculate size of male *Callosobruchus maculatus*

	Sex-linkage variance	
	Replicate 1 ($\times10^{-3}$)	Replicate 2 ($\times10^{-3}$)
Ejaculate size	2.01=42.0%	2.14=49.1%
Ejaculate size (proportion body size)	0.05=17.1%	0.06=31.1%

Variance components were estimated using the restricted maximum likelihood method of SAS VARCOMP. The sex-linkage variances (V_{LM}) were calculated assuming that maternal effects, dominance variance and higher-order interactions (e.g. V_{AA}, V_{AAA})=0. Calculations follow Becker (1984).

Table 3. Nested analyses of variance for mating duration of male and female *Callosobruchus maculatus*

	ANOVA			
Character/Source	*df*	*MS* ($\times10^{-2}$)	*F*	*P*
Replicate	1	0.13	0.04	0.843
Male's sire (rep)	29	3.08	0.91	0.601
Male's dam (sire, rep)	75	3.39	1.17	0.210
Female's sire (rep)	29	5.56	2.01	0.009
Female's dam (sire, rep)	70	2.77	0.96	0.575
Error	140	2.89		

Type III sums of squares were calculated using SAS GLM (SAS Institute 1985).

Roff 1997); for example, there was no evidence for dominance effects on body size in an earlier study of this species (Fox 1994) or in the seed beetle *Stator limbatus* (Fox 1998). For most life history traits, on the other hand, dominance effects can make a significant contribution, while behavioural and physiological traits, and probably ejaculate size, tend to be in between these extremes (Crnokrak & Roff 1995; Roff 1997). The effects of epistasis tend to contribute to the additive genetic variance term and as a consequence it is difficult to determine how much of the additive genetic variance is due to truly additive effects and how much is due to epistatic effects. Thus, it is not known how important epistatic effects generally are (Roff 1997). Although it has proven difficult to reliably estimate the number of loci that affect quantitative traits, it appears that for most such traits, the assumption of numerous loci of small effect is reasonable, with estimates ranging from the tens to hundreds of loci (Roff 1997).

Although a number of studies have demonstrated sex-linked loci that affect the viability of one or the other (usually heterogametic) sex of interspecific hybrids (reviewed in Charlesworth et al. 1987), there have been relatively few demonstrations of sex-linkage for other kinds of sex-specific traits, and most of these have focused on between-species differences rather than within-population variation. For example, between-species differences in male courtship songs of *Drosophila* are determined by loci on the X-chromosome (Bennet-Clark & Ewing 1970; Kawanishi & Watanabe 1981). Similarly, between-species differences in female mate preferences in *Colias* butterflies (Grula & Taylor 1980) and female oviposition differences in *Papilio* butterflies (Thompson 1988) are influenced by X-linked loci (since females are the heterogametic sex in butterflies, these traits are always hemizygous).

Table 4. Observational and genetic variance components (assuming autosomal inheritance) for mating duration of male and female *Callosobruchus maculatus*

		Variance components				
		Observational ($\times10^{-3}$)			Genetic ($\times10^{-3}$)	
		Replicate 1	Replicate 2		Replicate 1	Replicate 2
Males	V_S	0	0	V_A	0	0
	V_D	1.54	1.51	V_M	1.54	1.51
	V_E	37.23	24.44	V_e	37.23	24.44
Females	V_S	3.09	2.43	V_A	12.34=31.9±24.5%	9.71=36.8±34.5%
	V_D	0.91	2.28	V_M	0	0
	V_E	34.70	21.70	V_e	26.36	16.70

Variance components were estimated using the restricted maximum likelihood method of SAS VARCOMP (SAS Institute 1985). Genetic variance components V_A and V_M were calculated from separate models for males and females and for each replicate, with each model including only sire and dam (nested within sire) effects. The maternal effects variances (V_M) were calculated assuming no sex-linkage and that dominance variance and higher-order interactions (e.g. V_{AA}, V_{AAA})=0. Standard errors for the percentage V_P (phenotypic variance) explained by additive genetic variance (V_A) were calculated following Becker (1984). V_S=between-sire variance, V_D=between-dam variance, V_E=error variance in ANOVA, V_e=environmental variance. The heritability (h^2) of male mating duration was 0 in each replicate; h^2 of female mating duration was 0.32±0.25 and 0.37±0.35 for replicates 1 and 2, respectively.

Few examples of within-population genetic variation of sex-linked, sexually dimorphic traits are known. In guppies, *Poecilia reticulata*, within-population variation in the amount of orange colour on males is at least partially Y-linked (Houde 1992). In *Drosophila melanogaster*, selection experiments revealed that there is genetic variation, at least some of which is X-linked, in female traits that affect the latency to remate but not in male traits that affect remating interval (Pyle & Gromko 1981; Gromko & Newport 1988). If ejaculate size is in fact sex-linked in *C. maculatus*, then this species is unusual in exhibiting detectable within-population variation in a sex-linked trait. One possible explanation for the apparent scarcity of within-population genetic variation of sex-linked traits is that, since X-linked male-expressed traits are likely to evolve more rapidly than autosomal traits, they will rapidly evolve towards fixation and thus exhibit little genetic variation. It is not clear how genetic variation in ejaculate size is maintained in this population, if indeed it is sex-linked. It is possible that it is maintained by stabilizing or fluctuating selection: although large ejaculate size is favoured in the laboratory because females mated to males with large ejaculates do not remate as readily (Savalli & Fox 1998, in press) and may also be favoured by sperm competition (Eady 1995), in nature there sometimes may be selection for small ejaculates if males have many opportunities to remate and need to keep a supply of available sperm. Mutations and heterozygous advantage may also contribute to the maintenance of genetic variation (Roff 1997).

Although ejaculate size was correlated with male size, both absolute ejaculate size and relative ejaculate size (as a proportion of body mass) showed genetic variation. Thus, ejaculate size could evolve under stabilizing or counter selection on body size. The only other study, to our knowledge, to show heritable variation in paternal investment is Sakuluk & Smith's (1988) demonstration of heritable variation in the size of the spermatophylax in the cricket *Gryllodes supplicans*.

Although there was also genetic variation in copulation duration, only the female's sire, and neither the male's sire nor dam, affected copulation duration (i.e. there was genetic variation in the females' mating duration but not in the males' mating duration; Tables 3, 4). This suggests that copulation duration is under female control (which accords with behavioural observations). However, because females are homogametic, it is not possible from our experiment to determine whether mating duration is sex-linked or autosomal, although the lack of evidence for a genetic correlation between ejaculate size and mating duration suggests it is autosomal, assuming that ejaculate size is sex-linked.

The relatively weak negative relationship between ejaculate size and copulation duration, with ejaculate size explaining only 2–4% of the variation in copulation duration, was surprising, since we expected that it should take longer to transfer more material. Our result is consistent with a finding of little to no relationship between mating duration and ejaculate size within and among several species of *Drosophila* (Pitnick et al. 1991).

The evolutionary significance of heritable variation in copulation duration is not clear, since the weak relationship between copulation duration and ejaculate size was negative, contrary to our prediction. Why *C. maculatus* mate for the duration they do remains uncertain. One common explanation for mating duration is that it is a form of mate guarding (Alcock 1994), but mate guarding is unlikely to occur in *C. maculatus* since mating duration appears to be under female control and matings are short ($\approx$5 min) relative to the oviposition period of the female (multiple days). Another possibility is that mating duration is a component of postmating courtship (e.g. Eberhard 1996): females may require some initial period of mating to evaluate the male before they permit the transfer of ejaculate. High-quality males that are capable of producing large ejaculates may be able to initiate sperm transfer sooner than low-quality males. Alternatively, larger males, which produce larger ejaculates, may simply have larger sexual structures that permit sperm to be transferred at a higher rate, offsetting the increase in quantity.

There has been substantial interest in the evolution of male investments as well as in sperm competition (e.g. Clutton-Brock 1991; Birkhead & Møller 1992). Male ejaculate size can influence female fecundity and egg or offspring size and quality, and thus influence a male's fitness via nongenetic contributions to his offspring (e.g. Thornhill 1976; Thornhill & Alcock 1983; Ridley 1988; Fox et al. 1995b; Savalli & Fox 1998). 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 & Fox, in press). 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.

Nutrients within the male's ejaculate appear to be important to female *C. maculatus*: females that receive multiple ejaculates live longer, lay more eggs and lay larger eggs than once-mated females (Fox 1993a, b; Fox et al. 1995a, b). However, despite clear benefits from producing large ejaculates, few studies have demonstrated that there is heritable variation in this trait. Our study demonstrates that there is genetic variation in male investment via ejaculates, some of which is independent of body size. Our data suggest that some of the loci influencing ejaculate size are on the X-chromosome, such that ejaculate size may respond especially rapidly to selection.

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