# Temperature and host species affect nuptial gift size in a seed-feeding beetle

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**Summary**

**1.** In many insects species, males contribute large nutritional gifts to females during mating, generally as seminal fluids (ejaculates) or spermatophores. These nuptial gifts can affect both male and female fitness, and can mediate selection on male body size. However, it is unclear how environmental variables, such as temperature and diet, affect gift size and the consequences of gift size for male and female fitness.

**2.** We examine how temperature and rearing host affect male nuptial gift size (both total ejaculate size and the proportion of a male's mass allocated to his seminal fluids), and the relationship between gift size and female reproduction, in two populations of the seed-feeding beetle *Callosobruchus maculatus*.

**3.** Males reared at lower temperature (20 °C) produced substantially larger ejaculates than males reared at higher temperatures (25, 30 and 35 °C). However, males allocated a smaller proportion of their body mass to their ejaculate at the lowest temperature compared with other temperatures. This effect of temperature on male allocation to their ejaculates mirrored the effect of temperature on female body size – male ejaculate size remained a relatively constant proportion of their mate's body mass across temperatures.

**4.** Rearing host also affected male ejaculate size but the magnitude and direction of the host effect differed between populations.

**5.** Rearing temperature affected the relationship between male body mass and ejaculate size. Temperature also affected the relationship between female body mass and fecundity. The relationship between male body mass and ejaculate size was significantly lower when beetles were reared on cowpea than when beetles were reared on azuki or mung.

**6.** We found no evidence that male body size or nuptial gift size affected female fecundity in either population of *C. maculatus*. We thus propose that the effect of nuptial gift size on male fitness is through a reduction in female mating frequency and thus increased paternity for males producing larger nuptial gifts.

*Key-words*: *Callosobruchus maculatus*, ejaculate size, fecundity selection, phenotypic plasticity, spermatophore

## Introduction

In many insects, males contribute large nutrient packages to females during mating, often as seminal fluids (ejaculates), spermatophores or other nutritional gifts (Vahed 1998). The material in these nuptial gifts is generally incorporated into both female eggs and somatic tissues (Boggs & Gilbert 1979; Rooney & Lewis 1999; Wiklund *et al*. 1993). The size of the male's nuptial gift can have large effects on both male and female fitness

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(Wiklund & Kaitala 1995; Karlsson 1998; Rooney & Lewis 2002) and on female behaviour (Kaitala & Wiklund 1994; Wiklund & Kaitala 1995), imposing selection on male body size and influencing the evolution of sexual size dimorphism (Moya-Laraño & Fox 2006). The size and quality of these nuptial gifts, and thus their effect on male and female fitness, is often dependent on male size and physiological status (Simmons *et al*. 1999). Thus, environmental factors affecting male size and status can affect nuptial gift size and quality. Though effects of male age and mating history on gift size are well documented, only a few studies have examined environmental effects on nuptial gift

size or quality, including effects of food stress (Wedell 1994; Jia, Jiang & Sakaluk 2000) and parasitism (Lehmann & Lehmann 2000).

Two environmental variables of particular importance for the growth and development of herbivorous insects are diet (e.g. host species) and temperature. The effects of temperature are often substantial; in general, ectotherms that are raised at lower temperature mature later, are larger and lay larger eggs than those raised at higher temperature (Atkinson 1994; Fox & Czesak 2000), though there are many exceptions to these patterns (Stillwell & Fox 2005). Likewise, the effect of diet on adult phenotypes can be dramatic (Awmack & Leather 2002). Diet can affect male investment in spermatophores and seminal fluids (Simmons & Bailey 1990; Proctor 1992; Wedell 1994; Delisle & Bouchard 1995; Cook & Wedell 1996; Jia *et al*. 2000; Wagner 2005) but the effects of temperature on nuptial gift size, and the interactive effects of diet and temperature, have not been well explored.

In seed beetles (Coleoptera: Chrysomelidae: Bruchinae) males generally donate a large volume of seminal fluid to females during mating (Fox *et al*. 1995; Savalli & Fox 1998b; Takakura 1999). Substances in seed beetle ejaculates are incorporated into female somatic tissues and eggs (Das *et al*. 1980; Huignard 1983; Boucher & Huignard 1987). Behavioural data indicate that females treat these male nutrients as a food source – females with limited access to food, or access to only low-quality food, increase their mating rate (Takakura 2004). The size of male ejaculates also affects male fitness in at least two ways. In many species, females that receive larger ejaculates delay remating (have an increased refractory period) increasing the fertilization success of males that produce large ejaculates (Savalli & Fox 1998b; Takakura 2001) due to high second male sperm precedence (Eady 1995). In other species, females that receive large ejaculates have higher fecundity, which, when nuptial gift size is correlated to male body size (Fox *et al*. 1995), imposes fecundity selection on male size [(Fox & Czesak 2006; Savalli & Fox 1998b, 1999b) but see but see (Eady & Brown 2000)]. Females that mate more than once also experience various fitness benefits consistent with receiving material benefits from males; relative to once-mated females, females that mate multiple times have higher fecundity (Fox 1993b; Savalli & Fox 1999a; Wilson, Tufton & Eady 1999; but see Arnqvist, Nilsson & Katvala 2005), their egg size declines more slowly with age (Wasserman & Asami 1985; Fox 1993a), and they have extended adult lifespan (Fox 1993b, but see Savalli & Fox 1999a). Also, females that mate with nonvirgin males (which produce smaller ejaculates than virgin males) have lower fecundity and are more likely to remate than are females that mate to virgin males (Savalli & Fox 1999a).

It is thus clear that nuptial gifts affect both male and female fitness and, because nuptial gift size is correlated to male body size (Fox *et al*. 1995), they can mediate selection on male body size (Moya-Laraño & Fox 2006). However, it is unknown how environmental variables affect gift size and the consequences of gift size for male and female fitness. Here we examine how temperature and rearing host affect body size, male ejaculate size (both total ejaculate size and the proportion of a male's mass allocated to his seminal fluids), and the relationship between male ejaculate size and female reproduction, in the seed beetle *Callosobruchus maculatus*. Males of *C. maculatus* transfer ejaculates that represent > 5%, and sometimes more than 10%, of their body mass (Fox *et al*. 1995). Growth, development and female reproduction of *C. maculatus* are influenced by temperature and rearing host (Mookerjee & Chawla 1964; Lale & Vidal 2003; R.C. Stillwell, W.G. Wallin, L.J. Hitchcock & C.W. Fox, unpublished data), but no studies have examined how temperature and rearing host affect male nuptial gifts or the effects of those nuptial gifts on female reproduction.

## Methods

### STUDY POPULATIONS

The life cycle of *Callosobruchus maculatus* revolves around seeds of its host plant. Females cement their eggs to the surface of host seeds, particularly beans of the genus *Vigna*. First instar larvae burrow through the seed coat and into the seed. Larval development and pupation are completed within a single seed; they do not emerge until adulthood. Emerging adults mate and females begin to lay eggs within hours of emerging from the seed. Adults reproduce using primarily metabolic water and the resources acquired during larval development; i.e. they are capital breeders (Messina & Slade 1999). In some bruchids adult feeding on pollen, floral or extra-floral nectarines, or leaf fungi is common and these nutrients are used for maturation of eggs (Clement 1992). In *Callosobruchus* and some other storage pest bruchids access to adult resources has a small positive effect on female fecundity and improves adult lifespan (Leroi 1978; Shinoda & Yoshida 1987; Fox 1993a; Tatar & Carey 1995). However, *C. maculatus* adults have no access to food or water in a storage environment (they cannot feed externally on seeds) and there is little evidence that they feed as adults outside of a storage environment.

We examined ejaculate size and its effects on female fecundity in two populations of *C. maculatus* that were collected from and maintained on different legume hosts. The South India (SI) population was collected in 1979 from infested pods of Mung Bean, *Vigna radiata* (L.) Wilczek, and the closely related Black Gram, *V. mungo* (L.) Hepper, in Tirunelveli, India (Mitchell 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of Cowpea, *V. unguiculata* (L.) Walp., in Ouagadougou, Burkina Faso (Messina 1993). These two populations differ in body size, lifetime fecundity, patterns of egg dispersion, oviposition

preference, and adult longevity (Fox *et al.* 2004a; Fox, Czesak & Wallin 2004b; Messina 2004b). Both populations were maintained in laboratory growth chambers at ≈ 24–28 °C on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at > 1000 adults per generation for > 100 generations (BF) or > 200 generations (SI), prior to this experiment.

### EXPERIMENTAL DESIGN

Seeds bearing *C. maculatus* eggs were randomly selected from our laboratory colonies and isolated in 35 mm Petri dishes (one seed per dish, one egg per seed). Adults emerging from these seeds were randomly paired with a beetle of the opposite sex (within populations). These mated pairs were confined in a Petri dish with either 30 seeds of cowpea (*Vigna unguiculata*), 40 seeds of mung (*V. radiata*), or 30 seeds of azuki (*V. angularis*); pairs were not rotated among hosts. Dishes were checked for eggs twice per day until females laid eggs on 32 seeds (seeds bearing eggs were replaced at each check). Eggs were divided evenly among four rearing temperature treatments (20, 25, 30 and 35 °C). Average annual temperatures in their native collection localities are in the middle of this temperature range. Previous studies have found that 30–35 °C represent optimal temperatures for *C. maculatus* development (Mookerjee & Chawla 1964; Lale & Vidal 2003), and that eggs fail to develop at 40 °C (Lale & Vidal 2003).

These eggs were used for two separate experiments, only one of which is reported here; the companion experiment focuses on complex norms of reaction for a variety of growth and life-history traits (R.C. Stillwell *et al.* unpublished data). For the current experiment we randomly selected two hatched eggs per temperature per family. These larvae were reared to adulthood inside their host seed at their treatment temperature, LD 15 : 9 h, at one larva per seed, one seed per dish, in reach-in growth chambers. Dishes were checked every 12 h for emerging adult beetles. Upon emergence all beetles were transferred to a clean 35 mm Petri dish and into a new growth chamber at 27·5 °C, LD 15 : 9 h. Because males emerge with their ejaculate only partially formed (Fox *et al.* 1995) males were aged for 48 before mating. Males were then mated to a virgin female that was 24–36 h post-emergence and from the same population × temperature × rearing host combination.

Prior to mating all beetles were weighed to 0·01 mg precision. Because measurement error is large at this degree of precision, beetles were weighed two times or, if these first two measurements differed by > 0·03 mg, three times (in cases where one measurement was an extreme outlier that beetle was weighed a fourth time). Pairs were then allowed to copulate after which they were again weighed. We consider male mass loss during mating an estimate of male ejaculate size, and female mass gain during mating an estimate of the amount of ejaculate transferred to females during mat-ing; these two estimates are highly correlated but females tend to gain slightly less mass than males lose (Savalli & Fox 1998a).

After mating females were transferred to a 60-mm Petri dish containing ≈ 170 seeds of mung and allowed to lay eggs (at 27·5 °C, LD 15 : 9 h) until death. Our choice of oviposition host certainly affects female egg-laying behaviour and lifetime fecundity (Messina & Karren 2003) but our focus here is on the effects of larval environments on reproduction and not on the effects of variation in adult environmental experiences; we thus used only one adult oviposition host. Note also that this study confounds male and female rearing environments. Though it would be nice to disentangle effects on males from effects on females, the samples sizes needed to do so are impractical. Also, because males and females share identical larval environments it is probably generally the case, within populations, that host and temperature conditions are rarely inde-pendent between the sexes, such that our design reflects the most common natural situation.

In total, we collected adult body size and male ejaculate size data from 822 mated pairs, spread fairly evenly between population and among treatments. Of these, 747 females laid eggs.

### STATISTICAL ANALYSES

For most analyses of temperature and rearing host effects we used analysis of variance with Population, Temperature, and Rearing Host as fixed effects. For families that produced more than one male we used the average trait of siblings in our analysis (i.e. families are our lowest level of independence; *n* = 322 for BF and 311 for SI, relatively evenly distributed across treat-ments). When interactions including the population effect were statistically significant we ran partial ANOVAS, one for each population, to tease apart the nature of the interaction.

We used path analysis to examine relationships among traits and the effect of male ejaculate size on female reproduction. We first standardized all data to a Normal(0,1) distribution (within all population × treatment × rearing host combinations); i.e. we transformed all traits so that they had a mean of 0·0 and standard deviation of 1·0 within each population × treatment × rearing host combinations (24 com-binations in total). Note that this does *not* change the relationships among variables within each population × treatment combination but does remove the mean differences among treatments (including temperature × rearing host interactions). We then used the path analysis to compare path coefficients: (1) between populations; (2) among temperature treatments; and (3) among rearing hosts. Path coefficients are pre-sented in standard deviations; a coefficient of *r* for the path X → Y indicates that a change in X of 1 SD will produce a change in Y of *r* SDs. Only females that laid at least 10 eggs were used in the path analysis.
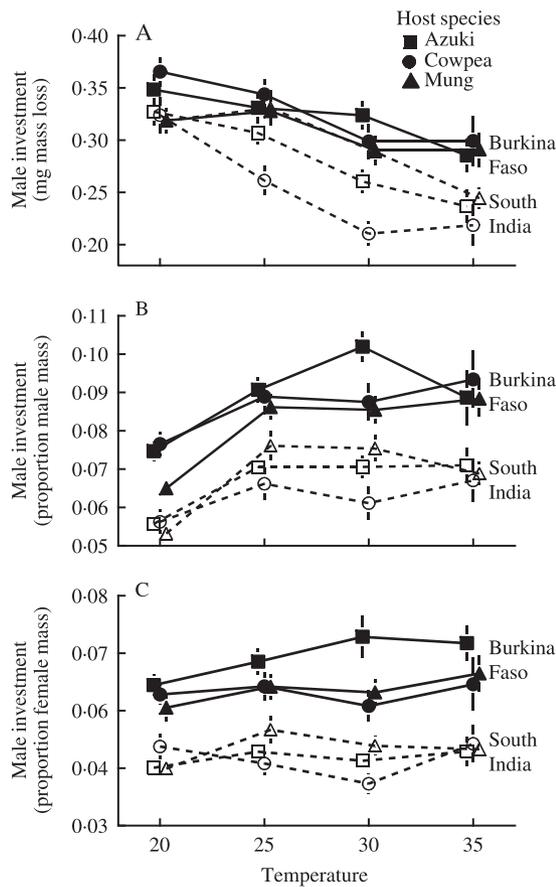
**Fig. 1.** Male ejaculate size (± standard errors) measured as (A) male mass loss during mating, (B) male mass loss as a proportion of male body mass, and (C) male mass loss as a proportion of his mate's body mass for *Callosobruchus maculatus* reared at four temperatures on three different host species.
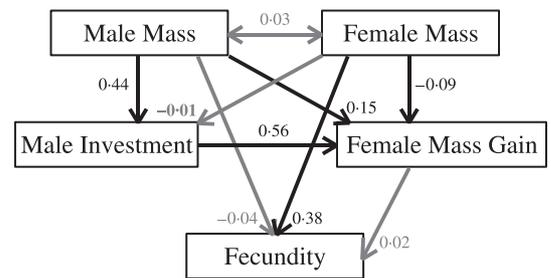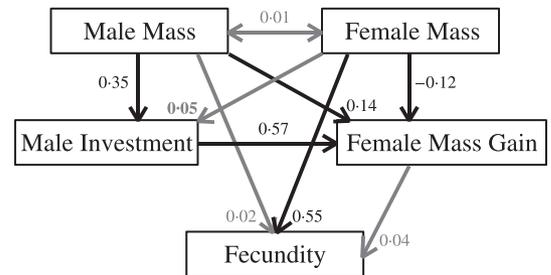


**Fig. 2.** Path analysis of the relationship among variables, and effects of male investment on female reproduction, for the BF and SI populations of *Callosobruchus maculatus*. To minimize clutter standard errors are not prevented; however, all bootstrap standard errors are less than 0·06. Black lines represent paths that are significantly different from 0 and grey lines are paths that are not significantly different from 0 based on whether 95% confidence limits overlap 0. Between population tests are presented in the text. The path model was fit using Amos 5·0 (Arbuckle & Wothke 1999).

## Results

### POPULATION, TEMPERATURE AND HOST EFFECTS ON NUPTIAL GIFT SIZE

SI males were ≈ 14% larger than BF males (least squares means, SI = 4·28 ± 0·03 mg, BF = 3·77 ± 0·03 mg; $F_{1,609}$ = 115, $P < 0.001$) but they produced ≈ 15% smaller ejaculates (LSMeans, SI = 0·278 ± 0·004 mg, BF = 0·318 ± 0·004 mg; Fig. 1A; $F_{1,609}$ = 44, $P < 0.001$) that represented an ≈ 30% smaller proportion of their body mass relative to BF males (LSMeans, SI = 6·6 ± 0·1%, BF = 8·6 ± 0·1%; Fig. 1B; $F_{1,609}$ = 115, $P < 0.001$).

Males reared at lower temperature produced larger ejaculates than beetles reared at higher temperatures (Fig. 1A; $F_{3,609}$ = 27·3, $P < 0.001$); males reared at 20 °C produced ejaculates that were 27% larger (LSMean, averaged between populations, 0·33 ± 0·01 mg) relative to the size of ejaculates produced by males reared at 35 °C (0·26 ± 0·01 mg). The temperature effect on ejaculate size was similar for both populations (Fig. 1A; nonsignificant temperature × population interaction, $F_{3,609}$ = 1·71, $P = 0.16$). Despite producing larger ejaculates at lower temperature, males of both populations allocated ≈ 25% less of their body mass to their ejaculate at 20 °C compared with beetles reared at any other temperature (Fig. 1B; LSMeans, 6·4 ± 0·1% at 20 °C vs. 8·0, 8·2, and 8·0% at 25, 30 and 35 °C, respectively; $F_{3,609}$ = 37, $P < 0.001$). Because sexual size dimorphism changes with temperature (R.C. Stillwell & C.W. Fox, unpublished data) we also examined how male allocation to their ejaculates was related to female size across temperatures. Intriguingly, the effect of temperature on male allocation to their ejaculates mirrored the effect of temperature on female body size; i.e. although males allocated a smaller proportion of their body mass to their ejaculates at low temperature, their ejaculate size remained a relatively constant proportion of their mate's body mass (4·6–5·1%) across temperatures (Fig. 1C; temperature effect on male ejaculate size as a proportion of his mate's body mass; $F_{3,609}$ = 2·54, $P = 0.06$).

The effect of rearing host on ejaculate size differed between populations (a significant population × host interaction; $F_{2,606}$ = 7·8, $P = 0.001$). SI beetles produced the largest ejaculates when reared on mung seeds (0·30 ± 0·01 mg) and the smallest ejaculates when reared on cowpea (0·25 ± 0·01 mg), a relative difference of 18% (Fig. 2A; $F_{2,310}$ = 7·8, $P < 0.001$). However, there was no significant effect of rearing host on ejaculate

size of BF beetles ($F_{2,321} = 1.87$, $P = 0.16$). After correcting for rearing host effects on male body mass (Fig. 1B) the host effect on ejaculate size disappeared for SI beetles ($F_{2,309} = 1.18$, $P = 0.31$) but became significant for BF beetles ($F_{2,319} = 6.56$, $P = 0.002$) – BF males produced the largest ejaculates (relative to their body size) when raised on azuki seeds ($9.02 \pm 0.02\%$ of their body mass) and the smallest ejaculates when raised on mung seeds ($8.21 \pm 0.02\%$). Interestingly, in contrast to our results for the effect of temperature, rearing host had a large effect on the size of the ejaculate relative to female body mass (ejaculate size/mate size) for the BF population (Fig. 1C) – when beetles were raised on azuki seeds the male ejaculate represented a much larger proportion (19% larger) of his mate's body mass than when beetles were raised on either of the other hosts ($F_{2,321} = 6.58$, $P = 0.002$). No such effect was observed in the SI population ($F_{2,310} = 1.15$, $P = 0.32$).

### WITHIN-TREATMENT RELATIONSHIPS AND THE EFFECTS OF MALE NUPTIAL GIFT SIZE ON FEMALE FECUNDITY

Figure 2 presents the path analysis showing the standardized path coefficients between body size, male ejaculate size and female fecundity for both the SI and BF populations of *C. maculatus*. This analysis removes the mean differences in trait values among treatments and averages the standardized regression slopes across all temperatures and rearing hosts. The first striking result is how similar the path coefficients are for BF and SI beetles. In fact, a Wald $\chi^2$ test comparing the two path models was nonsignificant ($\chi^2_8 = 9.8$, $P = 0.28$). However, examination of individual path coefficients detected two significant, but small, differences between populations. In both populations large females produced more eggs than small females, but female body size was a better predictor of fecundity in the SI (female mass $\rightarrow$ fecundity path; $r = 0.55 \pm 0.05$) than in the BF population (Wald $\chi^2_1 = 5.9$, $P = 0.01$). Also, although larger males produced larger ejaculates in both populations (male mass $\rightarrow$ male investment path) this relationship differed slightly between populations with male size a better predictor of male ejaculate size in the BF population ($r = 0.44 \pm 0.04$ for BF males, $r = 0.35 \pm 0.06$ for SI males; $P = 0.04$).

In both populations male investment was highly correlated to female mass gain (male investment $\rightarrow$ female mass gain path). However, female mass gain did not affect female fecundity in either population (female mass gain $\rightarrow$ fecundity path). Interestingly, female body size negatively affected female mass gain (female mass $\rightarrow$ female mass gain); i.e. large females gained less mass during mating than did small females ($r < -0.09$ in both populations). This was not because males produced smaller ejaculates when they mated to large females; there was no significant effect of female size on male investment (Fig. 2). Instead, a larger proportion of the male's ejaculate must be spilled or expelled when they mate with large females.

Analysis of covariance indicated that the factor having the largest effect on the path coefficients was rearing temperature, which affected both the relationship between male mass and male investment ($F_{3,708} = 3.2$, $P = 0.02$; $r$ between 0.33 and 0.48) and the relationship between female mass and female fecundity ($F_{3,708} = 12.8$, $P < 0.001$; $r$ between 0.11 at 20 °C and 0.72 at 35 °C). Pairwise Wald $\chi^2$ tests of all temperature treatment pairs indicated that the 20 °C treatment differed from all other temperature treatments in the structure of the path model ($\chi^2_8 > 30$, $P < 0.001$ for each comparison), whereas none of the other temperature treatments (25, 30 and 35 °C) differed from each other ($P > 0.05$ for each comparison). A comparison of individual parameters of the path model indicated that the path model for beetles reared at 20 °C differed from all other temperatures because female body mass was not correlated with female fecundity when beetles were reared at 20 °C ($r = 0.11 \pm 0.07$) but was highly correlated to fecundity in all other temperature treatments ($r = 0.52$ at 25 °C, 0.57 at 30 °C, and 0.72 at 35 °C).

Analysis of covariance also indicated that the relationship between male body size and male investment varied with rearing host (significant male mass × rearing host interaction, $F_{3,708} = 3.21$, $P = 0.02$) but there was no significant population × male mass × rearing host interaction ($P = 0.58$). Path analysis indicated that this was because the relationship between male body size and male ejaculate size was significantly lower when beetles were reared on cowpea ($r = 0.25 \pm 0.09$) than when beetles were reared on azuki or mung ($r = 0.45 \pm 0.05$ and $r = 0.45 \pm 0.06$, respectively) regardless of population. Rearing host did not significantly affect any of the other relationships in the path model.

## Discussion

The most significant results of this study are that: (1) both rearing temperature and rearing host have large effects on both the amount of material that males transfer to females during mating (the size of male nuptial gifts); (2) the relationship between male size and ejaculate size is affected by rearing host; (3) the relationship between female size and fecundity is affected by temperature; but (4) male ejaculate size has no detectable effect on female fecundity. We discuss each of these results below.

### EFFECTS OF TEMPERATURE ON NUPTIAL GIFT SIZE

Temperature affects adult body size of most (almost all) ectothermic animals (Atkinson 1994; Angilletta & Dunham 2003); insects are generally larger when reared at lower temperatures. It is thus not surprising that male ejaculate size, which is correlated with male body size, is largest when males are raised at low tem-

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perature. However, the proportion of a male's body size allocated to his ejaculate was actually smallest at low temperature. We know of only one other experiment examining temperature effects on male sperm or ejaculate production; in male Yellow Dung Flies (*Scathophaga stercoraria*) male testes (and thus, presumably, male sperm production) represent a larger proportion of a male's body mass when flies are reared at low temperature (Hellriegel & Blanckenhorn 2002; Blanckenhorn & Henseler 2005), opposite the pattern observed here. It is unknown if the effect of temperature on *C. maculatus* ejaculate size is mediated by an effect on testis or accessory gland size, and it is unknown whether the change in ejaculate volume reflects changes in sperm numbers, sperm size, or quantity of male accessory gland materials.

The largest effect of temperature on the proportion of a male's mass allocated to his ejaculate occurred between 20 and 25 °C (Fig. 1B). Possibly males experience substantial developmental stress at 20 °C and are unable to allocate as large a proportion of their biomass to ejaculate production at 20 °C relative to higher, less stressful temperatures. That the sex ratio of emerging adults becomes significantly female biased at 20 °C suggests that males are more sensitive to temperature and thus experience higher mortality than females when reared at low temperature. However, the effect of temperature on sex ratio is very small (47–49% male in the SI and BF populations, respectively, compared with 50–54% male at higher temperatures) (R.C. Stillwell & C.W. Fox, unpublished data). Also, although larval mortality increased about 5% at 20 °C relative to intermediate temperatures (25 and 30 °C), mortality is not higher when larvae are reared at 20 °C than at 35 °C (R.C. Stillwell *et al*. unpublished data), suggesting that although 20 °C is moderately more stressful for larvae than are intermediate temperatures, 20 °C is not more stressful for larvae than is 35 °C.

Rather than being a response to physiological stress encountered at low temperatures, we suggest that the change in male allocation to his ejaculate may be an adaptive response to temperature effects on sexual size dimorphism. Although the proportion of a male's body mass allocated to his ejaculate varied with temperature, male ejaculate size remained a fairly constant proportion of total female mass. This was because the effect of temperature on body size differs between males and females, such that sexual dimorphism varies with temperature (R.C. Stillwell & C.W. Fox, unpublished data). The effect of temperature on male ejaculate size mirrored the effect of temperature on female body size, and not the effect of temperature on male body size; as female size increased relative to male size (i.e. at low temperatures) male ejaculate sizes increased similarly. Note that within treatments males did not produce larger ejaculates when mating to larger females, indicating that the among-treatment relationship between ejaculate size and female size is unlikely to be a response by males to the size of the female with whom they are

in contact. Also, males are not responding to the temperature at which they are producing their ejaculate and mating with females – all males in our experiment were maintained at the same temperature while they matured their ejaculate, and all were mated at this same temperature, such that the only variation among males was their rearing temperature. Thus, males are likely responding to larval temperature by shifting the proportion of their body mass they allocate to reproductive tissues or their ejaculate. We suggest that this may be an adaptive response by males to ensure that they provide large enough ejaculates to reduce female remating. Previous studies with seed beetles have suggested that female remating behaviour is affected by male ejaculate size – females are more likely to remate after mating with small (Savalli & Fox 1998b) or nonvirgin (Savalli & Fox 1999a) males, both of which produce small ejaculates. Were ejaculate size to remain a constant proportion of male body size across all temperatures it would represent a smaller proportion of female size at low temperatures such that female behaviour, and thus the dynamics of sexual selection and sperm competition, would change with temperature. An alternative adaptive explanation may be that the reduced investment into ejaculates by males at low temperature is an adaptive response to temperature effects on male lifespan. Male life expectancy for insects typically increases at low temperature (Vieira *et al*. 2000). It may thus be beneficial for males to reduce investment in their early matings if their expectation of future mating success increases with their lifespan.

In contrast to these adaptive explanations it is possible that the change in the proportion of a male's mass allocated to his ejaculate reflects a nonisometric (i.e. allometric) relationship between male reproductive tissues and male body size, as observed for testis size of Yellow Dung Flies (Hellriegel & Blanckenhorn 2002). Egg size, ovariole number and female fecundity all change with temperature in a variety of insects (Stillwell & Fox 2005). These temperature effects are rarely isometric with body size such that female investment into reproduction as a proportion of female mass typically changes with temperature (Delpuech *et al*. 1995; Stillwell & Fox 2005). Unfortunately, we do not have the data in this current experiment to distinguish adaptive from nonadaptive explanations for the changing proportion of male size allocated to their ejaculates.

### REARING HOST EFFECTS ON EJACULATE SIZE

We found that larval diet affects ejaculate size in *C. maculatus*, though much of this effect was mediated through diet effects on body size. After removing the host effects on body size we found that larval diet affected ejaculate size only for the BF population – males produced the largest ejaculates when they had been raised on azuki beans and smallest when reared on mung seeds, though the effect varied with temperature. Though the host effect on ejaculate size relative to

male body size was fairly small, the effect on ejaculate size relative to female body size was quite large – ejaculate size was 14% larger (relative to female body mass) when males were reared on azuki compared with cowpea, and 12% larger when females were reared on azuki compared with mung (averaged across temperatures). It is unclear how much this host effect will influence female reproduction or behaviour, but we expect the effect to be biologically significant if female receptivity is controlled in part by stretch receptors in her spermatheca.

Multiple studies have examined the effects of food stress on nuptial gift size (Jia *et al*. 2000), quality (Wedell 1994), or rate of production (Wagner 2005). These studies all find that reduced food quantity or quality (e.g. protein content) reduce the size and quality of male nuptial gifts, or the rate of production of nuptial gifts. How the diet effect we observed for the BF population of *C. maculatus* relates to nutritional stress is unclear. BF beetles are adapted to seeds of cowpea (*V. unguiculata*), the host upon which they were originally collected and have been reared in the laboratory for > 100 generations. Recent experiments by Messina and colleagues (Messina & Karren 2003; Messina 2004a,b) demonstrate that beetles adapt to these hosts very quickly. It was thus unexpected that azuki seeds, rather than cowpea seeds, were the host that allowed beetles to produce the largest ejaculates, especially as this effect was not also seen in the SI population.

### RELATIONSHIPS AMONG TRAITS AND THE EFFECT OF MALE EJACULATE SIZE ON FEMALE REPRODUCTION

Because of the large effects of temperature and rearing host on body size, ejaculate size and fecundity, we had expected temperature and rearing host to have large effects on the relationships among these traits within treatments. However, we found that rearing environment had biologically significant effects on only two of the relationships. Host species affected the relationship between male body size and ejaculate size – the relationship was weakest when beetles were reared on cowpea ($r = 0.25$ vs. $r = 0.45$ on the other two hosts). Temperature affected the relationship between female size and fecundity – female size was highly correlated with fecundity at high temperature ($r = 0.72$ at 35 °C) but only weakly correlated to fecundity at low temperature ($r = 0.11$ at 20 °C). These changes in the relationship between body size and male ejaculate size or female fecundity mean that the fitness consequences of adult body size will necessarily vary with rearing host and temperature. For example, fecundity selection on female body size will necessarily decline with decreasing temperature, due to the reduced relationship between female size and fecundity at low temperature.

The consequences of male nuptial gifts for male fitness are unclear in *C. maculatus*. We found no effect of the male ejaculate size on female fecundity in this study, regardless of temperature or rearing host. This result contrasts with results for another seed beetle, *Stator limbatus*, in which the size of male nuptial gifts has a large effect on female fecundity, resulting in substantial fecundity selection on male body size (Moya-Laraño & Fox 2006). Indeed, fecundity selection on male body size is quite substantial in *S. limbatus* (Fox & Czesak 2006) and is likely the explanation for why males are larger than females in that species (Savalli & Fox 1998b). In contrast, males are smaller than females in *C. maculatus*. Our results here suggest that this difference between species may be due to a difference in fecundity selection – male *C. maculatus* experience little or no fecundity selection on their body size. However, previous studies have demonstrated that females readily accept additional mates after a brief refractory period (Fox & Hickman 1994) and additional matings increase female fecundity relative to mating only once (Fox 1993b; Savalli & Fox 1999a; Wilson *et al*. 1999; Eady, Wilson & Jackson 2000). This is usually interpreted as an effect of male nuptial gifts on female reproduction rather than a consequence of sperm limitation for once mated females. Our results suggest that either the effect on female fecundity of variation in the size of ejaculate received during a single mating is very small relative to the nutritional benefit of multiple mating or that the interpretation of previous studies is incorrect – females obtain little or no nutritional benefit from male ejaculates.

So why do males produce such large ejaculates? The most likely reason is that male investment influences his fitness via effects on female remating behaviour. Females remate quickly when their first copulation has been interrupted (Miyatake & Matsumura 2004). Also, female *C. maculatus* that mate with nonvirgin males remate sooner than do females mating with virgin males (Savalli & Fox 1999a); virgin males produce larger ejaculates than do nonvirgin males (Fox *et al*. 1995) and Savalli & Fox (1999a) have suggested that this difference in ejaculate size likely explains the difference in remating behaviour. We are currently testing the hypothesis that male ejaculate size directly affects female behaviour and thus that large ejaculates evolve as a mechanism for reducing female receptivity and thus increasing paternity.

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