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Ejaculate size, second male size, and moderate polyandry increase female fecundity in a seed beetle

Jordi Moya-Laraño[a,b] **and Charles W. Fox**[a]
[a]Department of Entomology, University of Kentucky, S-225 Agriculture Science Building-North, Lexington, KY 40546-0091, USA and [b]Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Cientificas, General Segura, 1, Almería, 04001-Almería, Spain

Several hypotheses have been proposed to explain the evolution of polyandry in species that provide nuptial gifts. When nuptial gifts are in the form of nutritional elements in the ejaculate and ejaculate size is correlated with male body size, females can accrue both direct (nutritional) and indirect (genetic) benefits from multiple mating. We examined remating decisions in females of the seed beetle *Stator limbatus* and, using path analysis, examined the effects of male body size on the size of his ejaculate, the amount of ejaculate that was successfully transferred to females, and the overall effect of these variables on female fecundity. Larger males produced larger ejaculates and consequently transferred a larger ejaculate to females, but the effects on female fecundity differed between the females' first and second mates. Both larger first and second males were able to transfer more of their ejaculate to females than were smaller males. Both the total amount of ejaculate transferred by these males and polyandry (number of matings) were positively correlated to female fecundity independently of each other. However, larger second males were more successful at stimulating female fecundity independently of how much ejaculate they transferred. We also provide evidence that females are choosy during their second mating opportunity. Both female choosiness and higher female investment after mating with larger second males suggest that females may benefit from both direct and indirect effects from multiple mating. We also conclude that male body size is under both directional fecundity selection and directional sexual selection. *Key words:* body size, directional selection, ejaculate nutritial benefits, nuptial gifts, polyandry, sexual size dimorphism. *[Behav Ecol 17:940–946 (2006)]*

Adaptive hypotheses for the evolution of polyandry focus on both direct and indirect benefits of multiple mating to females (Andersson 1994; Arnqvist and Nilsson 2000; Moller and Jennions 2001). Among the direct benefits that females can accrue are those that are nutritional, achieved mostly through male investment (nuptial gifts, e.g., prey, spermatophores, ejaculates; Vahed 1998). By providing females with nutritional material, males can increase their reproductive success by increasing the proportion of eggs that are fertilized by their own sperm (mating effort; Wickler 1985) and/or by directly increasing the fecundity of the fertilized female (paternal investment; Thornhill 1976). Indirect benefits are achieved when females mate preferentially with males that have heritable traits that increase either the (genetic) quality (good genes) or the future reproductive success of their offspring (Fisher 1930; Andersson 1994). Females can ensure these indirect benefits by cryptic choice of males, a form of postmating sexual selection that allows females to selectively fertilize their eggs with the sperm of preferred males (Eberhard 1996). This selection pressure from female choice may in turn favor higher male mating effort and may consequently explain the evolution of nutritional ejaculates (Eberhard and Cordero 1995). However, Arnqvist and Nilsson (2000) argued that females may have (co)evolved adaptations to metabolize male accessory substances that probably originated to prevent females from remating. Females can use these nutrients for somatic maintenance and/or reproduction. This would in turn select for large ejaculates in males, giving rise to sexually antagonistic coevolution. Direct and indirect evolutionary benefits can simultaneously impose selection on female mating behavior and male investment into ejaculates, making it difficult to disentangle to what degree a female's reproductive success, if any, is due to each of these 2 mechanisms (e.g., Fedorka and Mousseasu 2002).

Fecundity selection on female size is generally considered the most plausible explanation for why females are larger than males in most ectothermic animal species (Darwin 1871; Fairbairn 1997; but see Moya-Laraño et al. 2002). However, in animals in which males invest a relatively large proportion of their body mass as nuptial gifts, sexual size dimorphism can be reversed (males larger than females). When male investment increases the fecundity of females, fecundity selection may be acting on males as well as on females. In the seed beetle, *Stator limbatus* (Coleoptera: Chrysomelidae: Bruchinae), a species that provide nuptial gifts, males are larger than females. Selection for high paternal investment (ejaculate size) could have driven the evolution of larger body sizes in males relative to females. This is especially plausible in *S. limbatus* because body size is heritable (Fox et al. 1999), and ejaculate size is genetically correlated with body size (J Moya-Laraño, MET El-Sayyid, and CW Fox, unpublished data). Thus, this species is a good system to study the body size correlates of direct (nutritional) and indirect (genetic) effects of multiple mating. By weighing males and females before and after mating (Savalli and Fox 1998a; Edvardsson and Tregenza 2005), one can distinguish the size of the ejaculate invested by the males from the amount that is successfully transferred to females. The ejaculate size invested by males (or transferred to the females) can then be related to female mating behavior and female reproduction to assess direct nutritional benefits

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Address correspondence to J. Moya-Laraño. E-mail: jordi@eeza.csic.es.
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of multiple mating to the females. Studies with *S. limbatus* have demonstrated that male body size is correlated to both female fecundity (Fox et al. 1995) and female mating behavior (Savalli and Fox 1998a), but the mechanism underlying these relationships is unclear. Recent studies in another seed beetle, *Callosobruchus maculatus*, have raised doubts about the importance of male investment (Wilson et al. 1999; Eady and Brown 2000; Arnqvist et al. 2005; Edvardsson and Tregenza 2005). However, similar data are not available for *S. limbatus* in which male investment has been invoked as a major source of selection on male size (Savalli and Fox 1998a).

Here we use mating trials in the laboratory to test the hypotheses that 1) the size of the ejaculate invested by male *S. limbatus* has a direct effect on female fecundity, 2) body and/or ejaculate size of the female's first mate affect her decision whether to remate, 3) relatively larger males transfer more of the ejaculate that they invest to females, 4) moderate polyandry (mating twice) influences female fecundity due to the nutritional content of ejaculates, and 5) multiple-mated females or females mating with larger second males have higher fecundity regardless of the total ejaculate acquired during mating. This latter finding would be consistent with the hypothesis that females allocate more resources to the offspring of higher quality males (differential allocation: Burley 1988; Sheldon 2000) or, alternatively, with the hypothesis that males manipulate female reproduction (Colegrave 2001; Gil and Graves 2001).

METHODS

Study population

Beetles were collected from central Arizona, USA, as larvae inside the seeds of 2 common host species (*Acacia greggii* or *Parkinsonia florida*) and then maintained in the laboratory at >200 beetles per generation for at least 2 generations before the experiment started (to remove environmentally based maternal effects). Beetles are raised in 35-mm Petri dishes on seeds of *A. greggii* (because larval survival is high on this host) at ~28 °C, light:dark 16:8 h. All larvae are raised at one egg per seed, one seed per dish, such that all emerging beetles are virgin until provided with a mate. Details on colony maintenance are available in various other articles (Fox et al. 1999; Czesak and Fox 2003).

Experimental design

The methods in this experiment follow those of previous research (Savalli and Fox 1998a) with some minor modifications. In short, a virgin male and a virgin female of *S. limbatus* were paired in a 35-mm Petri dish with either an *A. greggii* seed or a *P. florida* seed (beetles mate more slowly when not exposed to host seeds). We checked the dishes once every 5 min (for 4 h) until the pairs mated. Most pairs that mated did so within the first 10 min of observations. After mating, females were provided 15 seeds of either *A. greggii* or *P. florida* (the same seed present during mating) and allowed to lay eggs for 24 h. Females were then paired with a second male randomly chosen from the pool of males that had been used in the first mating trails with the restriction that the second male could not be the same as the first. Thus, all males used in the first mating trials were virgin, whereas most males in second mating trials had mated once. Because this difference can affect the ejaculate size of males, we have included male mating status as a controlling variable in multivariate analyses and test for differences in investment between mated and virgin males. The second mating trials were performed exactly as were the first trials. After their second mating, females were confined in a 60-mm Petri dish with 20 seeds and allowed to lay eggs until death. More seeds were provided the second time because most eggs are laid after the first 24 h of oviposition (C Fox, personal observations).

All mating trials were performed in a Percival reach-in chamber at 28 °C between 12 and 6 PM (1200–1800 h) during the middle of the light cycle of a 16:8 h light:dark photoperiod. Multiple pairs were observed simultaneously in each of 5 blocks (each consisting of 50, 28, 19, 18, and 31 trials, respectively, depending on beetle availability; $N = 146$). For mating trials, we used beetles that had emerged from their host seed 24–48 h before mating to maximize the probability that they were mature, ready to mate, and of equal age.

Before and after each mating trial, both the male and female were weighed to the nearest 0.01 mg. Each beetle was weighed 2 or more times until the difference between the 2 measurements was <0.04 mg. We used the average among the 2 closest measurements as our estimate of mass. In most occasions (above 95%), weighing the beetles 2–3 times was enough to get 2 valid measurements. Henceforth, "male investment" refers to male mass loss during mating, and female mass gain (female gain) is treated as a measure of the amount of ejaculate actually transferred to females; these are correlated, but females generally gain less mass than males lose ($R^2 = 0.3$, $P < 0.0001$, $N = 94$, including all males that successfully mated and could be weighed before either dying or escaping, data for first matings in this study) either because females occasionally expel part of the male ejaculate or because females may prevent successful ejaculate transfer by the males. When beetles were weighed after mating, we noticed that some of the females (18% in first mating trials and 42% in second mating trials) had laid eggs (on average, one egg) during the 4-h period of observations. We counted these eggs and included them in our statistical analysis to correct for effects of oviposition on the change in female mass. We used the total number of eggs laid by a female as our estimate of fecundity (i.e., lifetime female fecundity). "Early fecundity" refers to eggs laid between the first and second matings, and "late fecundity" refers to eggs laid after the second mating trial.

Statistical analyses

We used generalized linear mixed models (GLMMs) with either normal (fecundity) or binomial (remating) distributions and a Type 3 hypothesis. This test compares by means of a log-likelihood ratio test (G^2) the model with the variable of interest against the model without that variable and has been shown to be more powerful than other methods of hypothesis testing in generalized linear model (GLM) (Agresti 2002). To allow the inclusion of categorical random (block) effects using binomial distributions, we used the macro "glimmix" in SAS (Littell et al. 1996).

The mating history of first and second mates is necessarily different by virtue of the experimental design (see above); all males used in the first mating were virgin, whereas most males (ca., 75%) used in the second mating trials had mated previously. Because male mating history per se could affect female behavior and fecundity, we included male mating status as a covariate in all multivariate models.

In those trials from block 1 in which the beetles successfully mated, we did not measure fecundity ($n = 20$ trials); block 1 data were thus only used for analyzing the female tendency to remate. Only females that had mated with their first male were used to test for the remating predisposition of females ($N = 99$ females). In the remaining 47 trials (from a total of 146), females did not mate, and we excluded them from further analysis. Previous work found no evidence of female choice in first matings (Savalli and Fox 1998a).

We used path analysis (Mitchell 1993; Tabachnick and Fidell 2001) to distinguish the direct and indirect effects of body size, ejaculate size, and ejaculate transfer of both first and second mates on female fecundity. Note that direct and indirect effects here are just "path effects" (whether the arrow in the path points straight to fecundity or reaches fecundity through some other variable) and not evolutionary direct (nutritional) or indirect (genetic) effects as discussed in the Introduction. Because most of the variation in female fecundity could be assigned to individual differences and not to block differences (see Results), we pooled the data for all blocks for the path diagrams. We performed 2 path analyses. The first path analysis was used to distinguish the effects of the first mate's body size, ejaculate size (male mass loss), and ejaculate transfer (female mass gain) on total female fecundity; this included all females that mated with at least their first partner. Excluding those trials from block 1, in which the beetles mated but fecundity was not measured ($n = 20$) and other trials in which some of the variables could not be measured either because the beetles died or flew away during the experiment ($n = 13$), we had a total of 66 females for this first path analysis. Because second males can influence female fecundity only after the second mating, the second analysis ($N = 45$ females that mated out of the 66 from the first path analysis) examined late fecundity (eggs laid after the second mating). In this analysis, we included only those females that mated twice (polyandrous). Also, because too few degrees of freedom were available to test for all possible effects, we treated female mass gain from first matings as an exogenous (controlling) variable. This model was built to test the hypothesis that larger second males would increase late female fecundity regardless of total ejaculate transferred to the females. Because some females laid a few eggs during the mating trial (see above), we included the number of eggs laid as a covariate (exogenous variables) in the path analysis. Also, because fecundity is higher when females are laying on *Acacia* seeds than on *Parkinsonia* seeds (Czesak and Fox 2003), we included seed type as an exogenous variable. These same 2 controlling variables were used in a GLMM analysis of female fecundity (with block effect as a random variable), which was run to disentangle the effects of total ejaculate size (from both males) from that of polyandry per se. To minimize confusion for readers, these covariate effects are not presented in the results. For this last GLMM analysis, the sample size included 63 of the 66 females included in the first path analysis (except for 3 cases in which either the males died or escaped before all the necessary measurements were taken). For the path analyses, we used AMOS 5.0 (Arbuckle and Wothke 1999; Arbuckle 2003).

Table 1

Variables measured during this study (means ± SEs are shown when appropriate; number in parenthesis denote sample size)

	First matings	Second matings
Number of trials	146	99
% Mated	68%	59%
Male mass (mg)	3.09 ± 0.06 (66)[a]	3.24 ± 0.08 (45)[b]
Female mass (mg)	2.95 ± 0.05 (66)[a]	3.05 ± 0.06 (45)[b]
Ejaculate mass (mg)	0.22 ± 0.01 (66)[a]	0.29 ± 0.02 (45)[b]
Ejaculate transferred (mg)	0.09 ± 0.01 (54)[c]	0.06 ± 0.02 (25)[c]
% Transferred[d]	42 ± 9 (54)[c]	14 ± 6 (25)[c]
Fecundity[e]	8.1 ± 1.4 (23)	16.5 ± 1.1 (48)

[a] Data from the first path diagram (see text)

[b] Data from the second path diagram (see text).

[c] Only females that laid no eggs during mating and thus allowed very accurate estimates of ejaculate transfer were used for these calculations.

[d] Percentage of invested ejaculate by a male that is successfully transferred to a female.

[e] Fecundity was measured as the total number of eggs laid by a female on her lifetime (lifetime fecundity). Fecundity for first matings refers to monandrous females and fecundity for second matings refers to polyandrous females.

RESULTS

Table 1 shows the results of first and second mating trials. Females that mated twice laid twice as many eggs (16.5 ± 1.1) as did females that mated only once (8.1 ± 1.4). Males invested more ejaculate (measured as male mass before and after mating) than was actually transferred to the females. During second matings, males were able to transfer only 14 ± 6% of the ejaculate invested, compared with a 42 ± 9% transfer during first matings.

Female tendency to remate

The pattern of female remating was complex. In brief, larger females were more prone to remate with larger second mates if these females had previously mated with a relatively small male. This pattern was independent of the size of the ejaculate transferred by the first male. Remating by small females was not affected by the phenotype of either their first or second mate.

The GLM model for female remating probability was highly significant (GLM, binomial distribution: $G^2_7 = 26.2$, $P < 0.001$, $N = 99$) with all body size effects (female body mass, first male body mass, and second male body mass) and their 2-way interactions significant (all $P < 0.05$, Table 2). Most importantly, the 3-way interaction was also significant ($P = 0.005$). However, the size of the ejaculate transferred by the first male (female mass gain) did not explain a female's decision to remate ($P = 0.160$). The inclusion of random block effects into the model showed that the block effect was not different from zero (estimate = 0.35; $P = 0.249$) and did not change the results qualitatively (all significant effects remained at $P < 0.05$ in a GLMM; ejaculate transfer: $P = 0.229$). To uncover the nature of the 3-way interaction, we first ran 2 separate models, one for large females (above average, >2.84 mg) and one for small females (<2.84 mg), including only first male and second male body sizes as independent variables. The model for large females was significant ($G^2_3 = 12.2$, $P = 0.007$) with a significant first male body mass × second male body mass interaction ($G^2_1 = 9.0$, $P = 0.003$). The model for small females was not significant ($G^2_3 = 4.0$, $P = 0.267$). This pattern could mean that large females were picky, whereas small females were too

Table 2

GLM on female tendency to remate (binomial distribution) in *Stator limbatus* ($N = 99$)

Variable	Estimate	SE	Chi-square	df	*P*
Intercept	179.2	67.0			
First male mass	**−59.0**	**22.1**	**8.4**	**1**	**0.004**
Second male mass	**−56.8**	**21.9**	**7.6**	**1**	**0.006**
Female mass	**−68.6**	**24.7**	**9.2**	**1**	**0.003**
First × second male mass	**18.1**	**7.0**	**7.4**	**1**	**0.007**
First × female mass	**22.3**	**8.1**	**9.1**	**1**	**0.003**
Second × female mass	**21.8**	**8.1**	**8.5**	**1**	**0.004**
Three-way interaction	**−6.9**	**2.6**	**8.0**	**1**	**0.005**
Female gain (previous mating)	−4.2	3.0	2.0	1	0.160

Test statistics are Type 3. df: degree of freedom. Significant values for target hypotheses (other than covariates) are in bold.

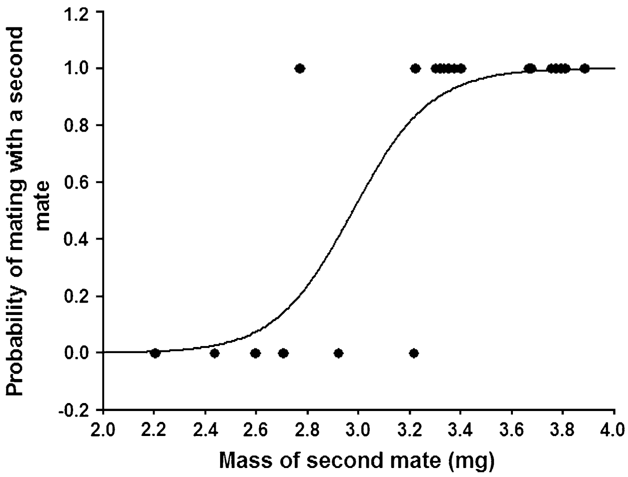

Figure 1
In the beetle *Stator limbatus*, large females (above average body mass; 2.84 mg) that had previously mated with relatively small males (below average body mass; 3.02 mg) were more likely to accept a second mate if the male was larger (logistic regression; analysis in the text, $N = 21$).

small to avoid harassment and choose the best males. If that were the case, we would expect more of the small females to remate relative to the large females, but the pattern was the opposite—21 out of 49 (43%) small females remated, whereas 36 out of 49 (73%) of the large females remated ($G_1^2 = 9.6$, $P = 0.002$). To examine the nature of the significant interaction, we further split the model for large females into 2 additional models, one for large first males (above average, >3.03 mg) and another for small first males (<3.03 mg), and included only the size of the second mate as the independent variable. The model for large first males was not significant ($G_1^2 = 1.2$, $P = 0.280$), whereas the model for small first males was highly significant ($G_1^2 = 15.4$, $P < 0.0001$), with larger second males increasing the probability of female remating (estimate ± SE = 6.6 ± 2.8, $R^2 = 0.52$, Figure 1). This pattern persisted ($G_1^2 = 16.1$, $P < 0.0001$) even after controlling for the mass of the ejaculate transferred by the first male (female gain), which was not significant in this model either ($G_1^2 = 1.4$, $P = 0.242$). This pattern of results did not change if we included the mating history of the male (i.e., virgin or mated with one female) as a covariate, which was not significant in any model (all $P > 0.15$).

Female fecundity

The first path diagram, in which we consider only the first mating by a female (Figure 2), reveals that male body size is positively correlated with male investment (mass loss during mating); that is, large males produce larger ejaculates, and females gain more mass during mating when males produce larger ejaculates. Interestingly, male body size (independent of male mass loss) explained the amount of mass gained by females during mating—females gain more mass when mated to larger males independent of the amount of ejaculate that males produce. In other words, larger males are more successful at transferring their ejaculate to females.

Female mass gain during mating affected female fecundity, but there was no direct effect of male size on female fecundity. Male body size affected female fecundity only indirectly; that is, through his effects on female mass gain during mating (Figure 2). These effects translated in a total positive effect of male body size on female fecundity (standardized path coefficient ± SE, $r = 0.18 \pm 0.07$).

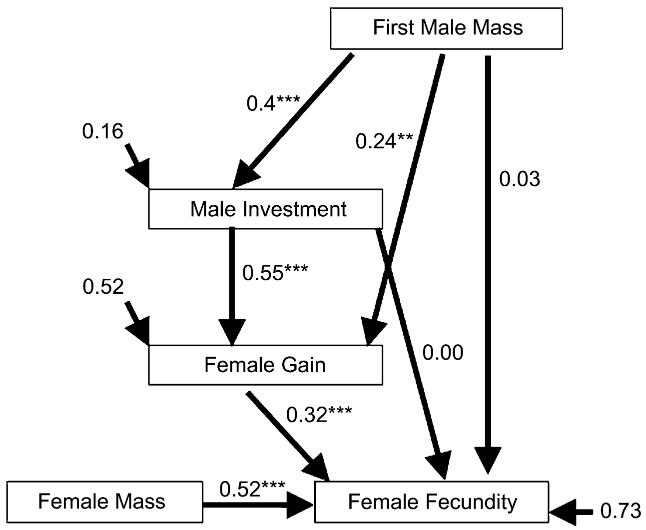

Figure 2
Path diagram testing the effects of first male body mass, first male ejaculate size (male investment), and size of the transferred ejaculate (female gain) on female fecundity in *Stator limbatus* ($N = 66$). Monandrous and polyandrous females were pooled. Both male size and investment positively affect female gain, which in turn affects fecundity positively. Female body size also affects fecundity. Solid lines denote positive effects. Numbers pointing to the variables are error variances.

The second path diagram shows the effects of the female's second mating on late female fecundity (Figure 3). The results are slightly different than in the previous path diagram.

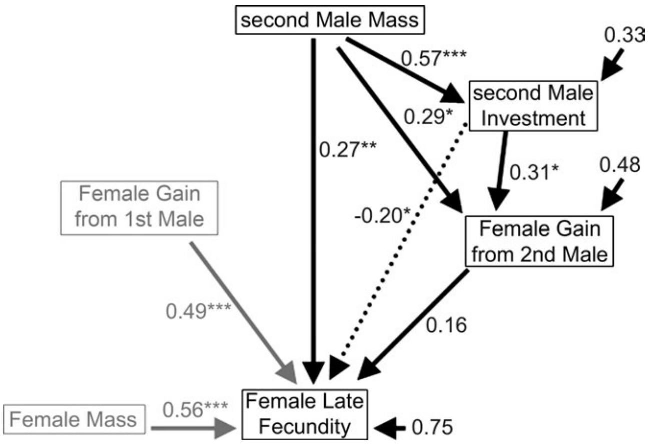

Figure 3
Path diagram testing the effects of second male body mass, second male ejaculate size (male investment), and size of the transferred ejaculate by the second male (female gain) on polyandrous female late fecundity of *Stator limbatus* ($N = 45$). The pattern is different than for first males. Male size positively affects female gain through male investment, but while male investment affects female late fecundity negatively, male body size directly affects female late fecundity. The overall path coefficient gives a positive effect of male body size on female fecundity (0.27), but the indirect effect is very different than for first males mating with both monandrous and polyandrous females. Solid lines denote positive effects, and dotted lines denote negative effects. Black color is used for hypothesized effects and gray color for covariate effects (i.e., those variables that were used to improve parameter estimation and power but for which no specific hypotheses were being tested). Numbers pointing to the variables are error variances.

Table 3

GLMM on female fecundity (normal distribution) in *Stator limbatus* ($N = 63$)

Variable	Estimate	SE	Z	df 1	df 2	F-value	P
Intercept	−0.7	0.4					
Block	1.8	2.5	0.7				0.238
First male mass	0.9	0.8		1	52	1.1	0.300
Second male mass	1.1	0.8		**1**	**52**	2.1	0.153
Female mass	5.9	0.9		1	52	43.9	<0.001
Polyandry	**2.8**	**1.0**		**1**	**52**	**7.7**	**0.008**
Total ejaculate mass[a]	**15.7**	**4.1**		**1**	**52**	**14.9**	**0.000**

Test statistics are Type 3. df: degree of freedom. Significant values for target hypotheses (other than covariates) are in bold.

[a] Total ejaculate mass refers to the total amount of ejaculate gained by females from either 1 or 2 matings.

As for first mates, both male investment (mass loss during mating) and male body size (independent of investment) explained the amount of mass gained by females during mating. However, male body size had a direct effect on female fecundity—females had higher late fecundity if they mated with larger second males ($P < 0.01$). Interestingly, despite larger males both investing more into reproduction and transferring more to females (second male mass → male investment → female gain; $P < 0.0001$ for each path), second male investment had a "negative" direct effect on late female fecundity ($P < 0.05$). This counteracted part of the direct effect of male size on fecundity such that overall positive effect on female late fecundity was 0.23 ± 0.09. Unlike for first matings, female mass gain from their second mating did not explain late fecundity ($r = 0.16 \pm 0.08$, $P = 0.18$). Thus, although male size had an overall positive effect on late female fecundity, this was not mediated by ejaculate transfer. Instead, though second male body size had a positive "direct" effect on female late fecundity, second male ejaculate size had a negative effect on female late fecundity, which translated into a small and nonsignificant negative "indirect" effect of body size on late fecundity ($r = -0.04 \pm 0.08$). This difference between the effects of first and second males could be because males were not all virgins for their second mating. However, ejaculate size and female fecundity were very similar whether females mated to a virgin or nonvirgin (mean ± SE, mated males [$n = 40$], ejaculate size = 0.30 ± 0.1, female fecundity = 12.3 ± 5.7; virgin males [$n = 5$], ejaculate size = 0.25 ± 0.1, female fecundity = 12.6 ± 0.9; Mann–Whitney U-tests, ejaculate size: $U = 82$, $P = 0.516$, female fecundity: $U = 87$, $P = 0.64$).

The GLMM showed that both total female gain during mating and female polyandry (whether the female mated with 1 or 2 males) explained female fecundity independently of each other (Table 3). Early fecundity (influenced only by the female's first male) was not correlated with late fecundity (influenced by both males) ($r = 0.03$; $P = 0.80$) even after correcting for female body size ($P = 0.53$).

DISCUSSION

Female remating propensity and relative ejaculate transfer: evidence for mate choice

Although the pattern is far from simple, our results are consistent with active mate choice by females. A significant 3-way interaction for female remating probability was disentangled using sequential analyses—only large females seemed to be selective of their mates, and within these females, choosiness was obvious only if females had mated with a small first male, after which they remated only if the second potential partner was large. This pattern was significant even after controlling for the amount of ejaculate transferred by their first mates, suggesting that remating is not a female strategy to hunt for nutrients for somatic maintenance and/or offspring production (Wedell and Karlsson 2003). Forced copulation by larger males is not a possible explanation either because remating with relatively larger males should be more frequent in small, not large, females. In addition, forced copulation did not occur in small females as they remated less frequently than did large females. Thus, females have control over mating even in the face of possible male harassment.

Mate choice seems to be a strategy by which females choose for relatively large males within the population. In *S. limbatus*, body size is heritable (Fox 1998). Thus, a large female mating with a male whose size is below average is likely to have small offspring. The question remains of why small females did not also prefer relatively larger second males when these females mated with a relatively small first male; only larger females showed this preference. Perhaps kicking during mating (Edvardsson and Tregenza 2005) allows relatively large (but not small) females to impose this sort of mate choice. Behavioral data are needed to test this hypothesis.

Both path analyses showed that larger males were able to transfer more of their invested ejaculate to the female. In other words, the amount of ejaculate gained by a female relative to that invested by the male (partial correlation) was higher for larger males. Because the amount of ejaculate gained by a female directly explains female fecundity (see below), this is an additional source of mate choice, suggesting that kicking may also play a role (i.e., active kicking by females may prevent small males from transferring a great proportion of their invested ejaculate). The hypothesis of a role for kicking in mate choice remains to be tested. However, males were able to transfer only 42% and 14% (first and second mates, respectively) to the females, also suggesting that selection for ejaculate transfer (and thus for male body size) is stronger during second matings. This last pattern is very interesting because in *C. maculatus*, (Savalli and Fox 1998b; Edvardsson and Tregenza 2005) above 90% of the ejaculate is successfully transferred to the female, suggesting stronger sexual selection for body size in male *S. limbatus* relative to male *C. maculatus*.

Two sources of mate choice (relative ejaculate transfer and female remating) along with the fact that body size is highly inheritable point to the direction that females accrue indirect benefits from good genes.

Ejaculate size and fecundity: fecundity selection in male *S. limbatus* and direct benefits to the females

Path analysis showed that the size of the ejaculate transferred to females by their first mate directly affected female fecundity. In addition, a GLMM showed that the total amount of ejaculate transferred by both the first and the second mate, and the number of times the female mated, affected female fecundity. To our knowledge, this is the first time that ejaculate size has been directly linked to female fecundity in a seed beetle. Previous research suggested that this would be the case in at least 2 different species of seed beetles (Fox 1993a, 1993b; Savalli and Fox 1998a, 1999), and evidence of multiple mating linked to higher fecundity exists for the seed beetle *Bruchidius dorsalis* (Takakura 1999). However, recent findings in *C. maculatus* contradict this hypothesis (Wilson et al. 1999; Eady and Brown 2000; Arnqvist et al. 2005; Edvardsson and Tregenza 2005). Wilson et al. (1999) suggested that the ejaculate of seed beetles serves as an oviposition stimulant (Eberhard 1996) rather than as a nutrient donation to increase female fecundity. However, they do not reject the second possibility. Until all the compounds in the ejaculates of seed beetles are identified (e.g., Das et al. 1980) and their function experimentally demonstrated, these hypotheses will be difficult to tease apart. However, we believe the relatively large percentage of a male's body mass allocated to ejaculates (5%; Savalli and Fox 1998a) is too large to reflect just oviposition stimulants. Also, numerous studies have demonstrated that materials in seed beetle ejaculates are incorporated into eggs of females (Huignard 1983; Boucher and Huignard 1987), suggesting a nutritional function of these ejaculates. Arnqvist et al. (2005) hypothesized that ejaculate benefits may include both hydration (from the water contained in the ejaculates) and true nutritional effects, both of which have been shown to affect fitness components in seed beetles (Leroi 1981). *Stator limbatus* is a desert beetle that reproduces in the middle of the summer when high temperatures and dehydration can be important. Females obtain water directly from the seed in which they grow, which may not be enough for successfully developing all her ova. Thus, hydration benefits from male donations are plausible. Offspring could also benefit from male nutritional investment in the form of substances that could allow the larval stages to penetrate the resistant coat of one of their hosts (*Parkinsonia florida*), which is a limiting factor for offspring development success (Czesak and Fox 2003). Whatever the mechanistic explanation, our results suggest that females accrue direct benefits from mating in the form of ejaculate nutrition.

The fact that body size is genetically correlated with ejaculate size (J Moya-Laraño, MET El-Sayyid, and CW Fox, unpublished data) and that higher ejaculate investment translates into higher female fecundity strongly suggest that male body size is under fecundity selection. Interestingly, sexual size dimorphism (SSD) in *S. limbatus* is reversed relative to most insects—males are larger than females. *Callosobruchus maculatus* exhibit the normal pattern for insects with males being much smaller than females. We interpret this difference as evidence that both sexual selection and fecundity selection affect the evolution of male body size in *S. limbatus* (Savalli and Fox 1998a), but only sexual selection seems to affect *C. maculatus*. Because ejaculate transfer is also under stronger selection in *S. limbatus* (see the above section), this difference in SSD between the 2 species could also be more pronounced due to stronger sexual selection in male *S. limbatus*.

Male body size, polyandry, and fecundity: male manipulation and/or female differential allocation?

In addition to investing in large ejaculates, male body size had other effects on female fecundity. During first matings, larger males were able to transfer a relatively larger proportion of their ejaculate to females. In polyandrous females, second male body size had a direct positive effect on female late fecundity, but the size of his ejaculate had a negative effect on female late fecundity. However, the overall effect of second male body size on female late fecundity was positive. Interestingly, polyandry alone, independently of total ejaculate transfer (from both males), had a positive effect on female fecundity. Excluding the nonsignificant block effects (model in Table 3) and running different regression models (with or without the effects), we calculated the different contributions of polyandry, ejaculate mass, and the joint (shared) contribution of both variables to female fecundity. Both variables contributed a 24.5% of the total variance in female fecundity. Polyandry alone only contributed 2.6%, ejaculate mass alone 11.6%, and the shared contribution was 10.3%. Because polyandry does not occur randomly, but is the result of female choice, it is possible that females are investing in higher fecundity due to the relatively higher quality of their second partners (the differential allocation hypothesis: Burley 1988). However, these effects of males on female fecundity may also be due to male manipulation (Holland and Rice 1998; Colegrave 2001; Gil and Graves 2001) possibly achieved via oviposition stimulants in the ejaculates or male physical interactions with the female (e.g., stroking the back of the female with their antennae, Rup 1986). The rate of antennal stroking has been shown to increase the chances that males are accepted as mates in another chrysomelid beetle (Tallamy et al. 2002). At this point, we do not know if male *S. limbatus* possess aedeagus spines that could aid in female manipulation; such spines have been demonstrated in another seed beetle (Crudgington and Siva-Jothy 2000). Alternatively, differential allocation by females (Burley 1988; Sheldon 2000) could also explain the effects of male size on female fecundity. Because we have evidence that females are choosy, it is not unreasonable to hypothesize that females will invest more into offspring sired by high-quality (genetically larger) males. One requirement for differential allocation to work is that there is a trade-off between present and future reproduction (Sheldon 2000) such that females that allocate a relative high proportion of their reproductive effort to current high-quality males risk lower reproductive success in the future. In this species, egg size is highly plastic (Fox et al. 1997; Czesak and Fox 2003); females mating with second high-quality males could potentially shift egg size to manipulate fecundity in response to male quality. Unfortunately, we did not measure egg size or sperm precedence in this study. Therefore, our results cannot conclusively distinguish between both hypotheses. Future experiments in which egg size and paternity were measured could provide a good test of the differential allocation hypothesis.

CONCLUSION

Polyandry in female *S. limbatus* may involve both direct and indirect beneficial effects to females. The ejaculate size obtained by females in their first mating directly affects female fecundity, and large females mated to small males are more likely to remate with large males, an effect that is independent of the amount of ejaculate gained from their first mating. This suggests female mate choice for good genes that confer larger body size. Also, larger males are able to transfer more of their ejaculate to the females also suggesting some form of mate choice for good genes by females. Thus, male body size is under strong sexual selection from mate choice and under fecundity selection.

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