The effects of male phenotypic condition on reproductive output in a sex role-reversed beetle

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ARTICLE INFO

In insects with sex role reversal in mating, in which females actively court males, large and nutritious ejaculates are a common direct benefit to females. Such ejaculates are costly for males to produce and their size and composition can depend on male condition. However, the fitness effects to males and females of such condition-dependent provisioning are less clear. Here, we studied the effects of phenotypic condition on mating behaviour, ejaculate size and reproductive output in honeylocust beetles, *Megabruchidius dorsalis*. Our experimental design allowed us to disentangle the independent effects of juvenile resource acquisition in both sexes (as reflected by body size) and resource acquisition by adult males (feeding). We show that phenotypic condition of both sexes had sizeable independent and interactive effects on mating and reproductive output. In males, resources accrued during the juvenile phase had significant but relatively marginal effects on male mating and reproduction. Male adult feeding, in contrast, had sizeable effects on almost all aspects of male and female reproduction, through the nutritional effects of ejaculates in females. We discuss our findings in light of the reversal of both sex roles and sexual size dimorphism exhibited by this species, relative to related species. Our results highlight the importance of testing the interaction of male and female condition on components of fitness to understand the evolution and maintenance of mating systems.

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In most animal species, sexual selection is stronger in males than in females as an indirect consequence of anisogamy (Bateman, 1948; Schärer, Rowe, & Arnqvist, 2012). Females produce large, costly eggs, which are typically produced at a slower rate than smaller, less costly sperm (Hayward & Gillooly, 2011). The reproductive rate of females is thus typically limited by egg production, while males are often limited by the number of eggs they fertilize (Bateman, 1948; Trivers, 1972). In contrast to this typical scenario, stronger sexual selection in females can occur when males provide females with substantial direct benefits, most commonly in the form of parental care, nuptial gifts or access to territories (Berglund & Rosenqvist, 1993; Fritzsche & Arnqvist, 2013; Gwynne, 1986; Takakura, 1999; Vahed, 1998). In this case, a male's reproductive success may not primarily be limited by his mating rate, but rather by his ability to provide such benefits (Proctor, 1992; Simmons, 1995; Simmons & Kvarnemo, 1997).

Large, nutritious ejaculates are a common direct benefit to females in insects (Choe & Crespi, 1997; Thornhill & Alcock, 1983). These ejaculates are costly for males to produce and their size and composition can depend on male condition (Boggs, 1990; Dewsbury, 1982; Gwynne, 1984, 1988; Moya-Larano & Fox, 2006; Reinhardt, Naylor, & Siva-Jothy, 2009; Thornhill, 1976; Ursprung, den Hollander, & Gwynne, 2009). Two main factors have been shown to influence the characteristics of such ejaculates. First, ejaculate size and composition often increase with male body size, analogously to the effects of size on fecundity in females (Jia, Jiang, & Sakaluk, 2000). Second, ejaculates can be affected by environmental conditions, particularly the availability and quality of food resources (Gwynne & Simmons, 1990; Perry & Rowe, 2010; Proctor, 1992). In katydids, for example, food shortage results in fewer sexually active males and smaller nutritious spermatophores among those males (Gwynne, 1993; Jia et al., 2000).

Male provisioning of direct benefits can select for multiple mating in females (polyandry) and for female choice of males that provide greater benefits. This can subsequently generate selection on males to provide greater benefits in order to attract females or to induce resistance to further mating in females (Arnqvist & Nilsson, 2000; Gwynne, 1990; Jennions & Petrie, 2000; Simmons, 2005;

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Simmons & Bailey, 1990; Wedell, 1996). Female choice for large males has been documented in several gift-giving insects. For example, female choice for large males in ground crickets generates direct sexual selection on male body size (Fedorka & Mousseau, 2002a). Males may thus evolve to exceed females in body size in gift-giving taxa (Bonduriansky, Wheeler, & Rowe, 2005), even in groups such as insects in which females are normally larger than males (Savalli & Fox, 1999; Ursprung et al., 2009).

However, the effects of male condition-dependent ejaculate characteristics on male and female fitness are not well understood (Fedorka & Mousseau, 2002a; Perry & Rowe, 2010). To study strategic ejaculate allocation, some studies have manipulated female condition and investigated the effects on male ejaculates and on females only (Bonduriansky et al., 2005; Fox, 1993; Takakura, 2004). Other studies have manipulated male condition but have focused strictly on ejaculate traits rather than male reproductive output (Perry & Rowe, 2010; Proctor, 1992). In this study, we aimed to provide a complementary contribution by investigating how the availability of food to males interacts with male and female body size to determine mating behaviour and reproductive outcome for both sexes in a role-reversed and gift-giving mating system.

In the sex role-reversed honeylocust beetle, *Megabruchidius dorsalis* (Bruchinae), males transfer an ejaculate that comprises some 5–12% of their body weight, which exceeds that in related seed beetle species (Rönn, Katvala, & Arnqvist, 2008; Takakura, 1999). Mating carries substantial costs to males (Salehialavi, Fritzsche, & Arnqvist, 2011). In contrast, females gain substantial direct benefits from mating and female offspring production increases with each additional mating (Takakura, 1999). Females have evolved to actively court males (Fritzsche & Arnqvist, 2013; Salehialavi et al., 2011; Takakura, 1999, 2006). Previous work has shown that male condition markedly affects the magnitude of direct benefits to females. Females mated to well-fed males lay larger eggs, produce offspring that perform better, and show reduced feeding behaviour in comparison to females mated with poorly fed males (Takakura, 2004). Females also prefer to mate with larger males, suggesting that direct benefits are larger when mating with large males (Salehialavi et al., 2011; Takakura, 2004).

Here, we assessed how body size of both females and males interacts with adult male food provisioning to influence mating success and reproductive output in this sex role-reversed mating system. We hypothesized that male size is under fecundity selection and that the nutritional value of a male's ejaculate is condition dependent, such that ejaculate weight is influenced by both juvenile and adult resource acquisition. Our design allowed us to separate effects of male resource acquisition during the juvenile phase (manifested as adult body size) from those that result from male resource acquisition during the adult stage. We predicted that (1) larger and better-fed males would transfer larger and more nutritious ejaculates and (2) females mated to such males would produce more offspring than those mated to small and/or poorly fed males, resulting in higher reproductive success for both these females and their mates, (3) mating behaviour of both sexes would depend on male size and feeding status and (4) the absolute reproductive benefits to females from receiving nutritious ejaculates should depend on female body size.

METHODS

Model Organism and Experimental Design

Honeylocust beetles were kept on seeds of *Gleditsia triacanthos* in 1-litre glass containers in climate chambers set to 26 °C, 70 ± 10%

relative humidity with a 16:8 h light:dark cycle. Under these conditions generation times are approximately 7 weeks. Virgin individuals were obtained by isolating single beans, each containing a single larva, in 24-well culture plates. Individual beetles were collected on 9 consecutive days directly after hatching and kept in petri dishes in same-sex groups (10 individuals).

All beetles were then weighed to the nearest μg using a microbalance (Sartorius Genius ME 235P). Individuals of both sexes were then selected from two weight classes: 'large' individuals were those in the upper 30 percentile of the weight distribution for their sex and 'small' individuals were those in the lower 30 percentile of the weight distribution. Individuals of each weight class and feeding treatment (for males; see below) were then randomly assigned to one of eight different mating treatments using a fully crossed $2 \times 2 \times 2$ design with nine or 10 replicates per cell. The three factors were male food treatment (fed versus nonfed), male size (large versus small) and female size (large versus small). In total 77 pairs of beetles were observed in repeated mating trials. To assess virgin life span, additional virgin individuals of both sexes were kept separately in petri dishes, replicated 8–10 times for each food treatment and size group.

To assess the effects of food provisioning to adult males, half of all males in each size class were provided with water, 20% sucrose solution and pollen (Bee Pollen Capsules, Manuka Health Ltd., New Zealand) while the other half were provided with water only. All females were provided with water only. We refer to the two male groups as the nonfed and fed male treatments, respectively. This feeding regime was maintained until the onset of the experiments (16 days). After the start of the mating experiment, all beetles were provided with water only.

Mating Trials

Our primary goal was to measure reproductive productivity of each male–female pair. Each pair was thus placed in a 3 cm petri dish and observed until a mating was completed, or for a maximum of 30 min if no mating commenced during this time. Pairs were then separated. The same pair was placed together again for a second trial after 24 h and a third trial after 48 h. The time period between matings was chosen to match the period males need in order to replenish their ejaculate (Salehialavi et al., 2011). Thus each pair could mate a maximum of three times over 3 consecutive days. To estimate the amount of absolute and relative ejaculate transferred during mating, both males and females were weighed immediately before and after each mating. Absolute ejaculate weight was calculated as the difference in male body weight before and after mating and relative ejaculate weight was calculated by dividing absolute ejaculate weight by body weight before mating.

In between and after mating trials, males were kept individually in plastic petri dishes and provided with water. Females were kept individually in glass petri dishes (12 cm) provided with water and 100 g of *Gleditsia* beans as an ad libitum substrate for oviposition. Larvae bore into a bean upon hatching, where they complete their development and emerge as adults. After the third mating trial, females and males were kept in their individual petri dishes until death and their life span was recorded. The beans in the female petri dishes were subsequently incubated in climate chambers until all offspring hatched and the number of offspring of each pair was recorded, as an estimate of their joint reproductive output.

Ethical Note

The honeylocust beetles used to conduct this experiment originated from field samples taken at Inogashira Park, Tokyo, Japan.

The beetles were kept on seeds of their host plant, *G. triacanthos*. The ancestral population was provided with ad libitum access to distilled water, 20% sucrose solution and pollen (Bee Pollen Capsules, Manuka Health Ltd., New Zealand), resembling natural conditions. All individuals in this experiment were handled with care and died of natural causes.

Statistics

The results were analysed using general linear fixed-effects or mixed-effects models, using type III sums of squares. The residuals of these models were tested for normality and homoscedasticity and fulfilled model assumptions in all cases, following log transformation of reproductive output. Models of ejaculate weight were estimated as mixed linear models (REML) with pair ID included as a random-effects factor to account for the fact that there were multiple measurements per pair. The variance component estimates for pair ID, however, are not given here. In models of reproductive output and life span, we also included number of matings as a factor. Since number of matings represents a count variable, variation in this variable was analysed in a generalized linear model with a Poisson error distribution and a log link function. Our inferential models included the main effects of all factorial variables and covariates, as well as those interactions that significantly ($\alpha = 0.05$) improved model fit to data.

## RESULTS

Total Number of Matings

Of 77 pairs, seven did not mate at all, 17 mated once, 13 twice and 40 three times. We found no main effects of male feeding or the size of either of the mates on the number of matings each pair performed (Table 1, Fig. 1). However, there was an interaction between male feeding regime and body size, such that small males mated less when not fed while large males did not show this reduction when deprived of food.

Ejaculate Weight

Large males and well-fed males transferred substantially larger ejaculates than did nonfed males (Table 2) and absolute ejaculate size decreased less over consecutive matings in fed males (Fig. 2). Further, fed males transferred a larger fraction of their body weight to females at mating and this decreased less rapidly over matings (Table 2). In total, well-fed males transferred 0.52 mg (54%) more ejaculate resources than nonfed males. Female size did not significantly affect the amount of ejaculate transferred, and was thus dropped from our inferential model. Interestingly, even though well-fed males of both sizes transferred significantly larger ejaculates, this only translated into significantly higher reproductive output for small males (see below).

Table 1
Analysis of variance of the effects of body size and male adult feeding regime on the total number of matings

Source	df	Wald-χ^2	P
Male size	1	2.28	0.123
Female size	1	0.25	0.615
Feeding regime	1	1.92	0.166
Male size∗Feeding regime	1	4.51	0.034
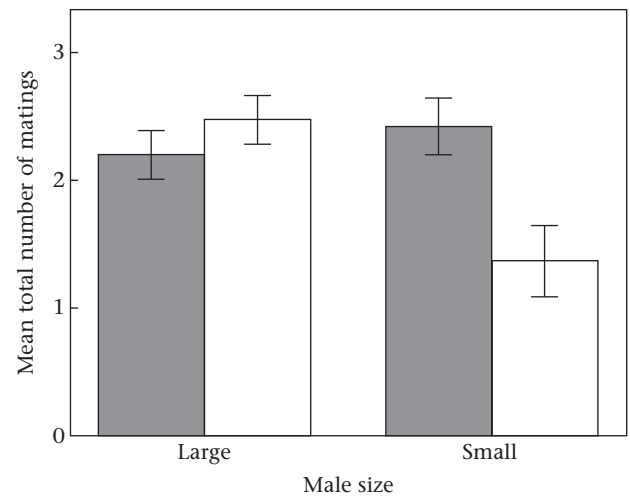
Figure 1. Mean ± SE total number of matings of large and small males in relation to whether they were fed (grey bar) or nonfed (white bar).

Consequences of Mating

Reproductive output

Across all pairs, reproductive productivity increased with the number of matings performed and this increase was stronger for well-fed than nonfed males (Table 3). Male and female size had independent main effects on offspring production, such that large individuals produced more offspring (Fig. 3a, b), but size also interacted with food treatment in both sexes. In males, feeding had a much stronger effect in small males. In females, in contrast, the largest effect of male feeding was seen in large females (Fig. 3). Thus, while large females produced more offspring than did small females, this effect was stronger when mated to well-fed males.

Male life span

Male life span increased with both male body size and food availability (Table 3). Large males lived slightly longer than did small males (large male: 31.14 ± 1.46 days; small male: 27.24 ± 1.28 days) and well-fed males lived on average 12 days longer than nonfed males (nonfed male: 23.08 ± 0.59 days; fed male: 35.53 ± 1.24 days).

Female life span

For females, life span was primarily influenced by the number of matings performed (Table 3, Fig. 3c). Once-mated females had slightly shorter life spans than virgin females, but life span

Table 2
Fixed effects from mixed-model analyses of variance/covariance of the effects of body size and male feeding regime on ejaculate weight

	Source	ndf	ddf	F	P
Absolute ejaculate weight	Male size	1	65.45	30.38	<0.001
	Feeding regime	1	67.49	53.52	<0.001
	Mating no.	2	97.40	32.40	<0.001
	Mating no.∗Feeding regime	2	97.15	11.92	<0.001
Relative ejaculate weight	Male size	1	66.84	2.65	0.108
	Feeding regime	1	68.87	46.56	<0.001
	Mating no.	2	99.27	33.74	<0.001
	Mating no.∗Feeding regime	2	99.03	14.36	<0.001

Mating no. refers to mating number (first, second or third). *ndf/ddf*: numerator and denominator degrees of freedom.
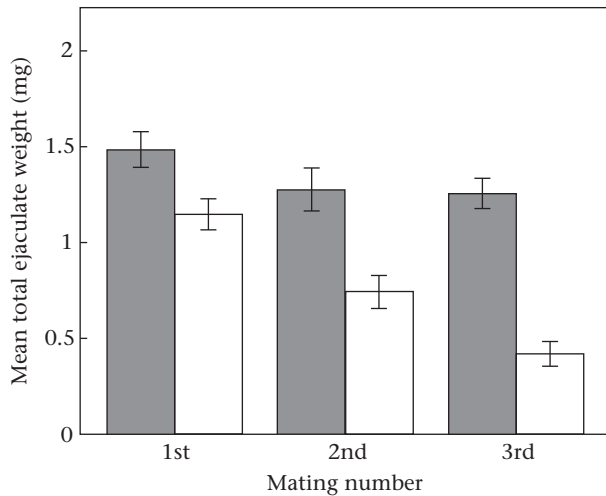
Figure 2. Mean ± SE total ejaculate weight of males over consecutive matings in relation to whether they were fed (grey bar) or nonfed (white bar).

increased with each further mating, such that females with three matings showed the longest average life span.

DISCUSSION

Our experimental design allowed us to disentangle the independent effects of adult resource provisioning (for males) and body size (of both sexes) on mating behaviour, ejaculate size and reproductive output. Below, we discuss the implications of our findings for males and females, separately.

The Male Perspective: Size and Nutrition

Our results show that large body size and high adult food availability are both beneficial to males. On average, well-fed males (1) mated more often, (2) transferred larger ejaculates, (3) produced more offspring and (4) lived longer than nonfed males. Interestingly, however, the larger ejaculates that were associated with higher food availability only translated into a higher

Table 3
Analyses of variance/covariance of the effects of body size and male feeding regime on reproductive performance and life span

	Source	ndf	ddf	F	P
Reproductive output	Male size	1	58	7.08	0.010
	Female size	1	58	9.67	0.003
	Feeding regime	1	58	0.28	0.601
	Total no. of matings	2	58	3.46	0.038
	Male size∗Feeding regime	1	58	6.73	0.012
	Female size∗Feeding regime	1	58	10.25	0.002
	Total no. of matings∗Feeding regime	2	58	4.01	0.023
Female life span	Male size	1	69	1.94	0.168
	Female size	1	69	0.14	0.711
	Feeding regime	1	69	0.93	0.337
	Total no. of matings	3	69	10.50	<0.001
Male life span	Male size	1	67	8.04	0.006
	Female size	1	67	0.68	0.414
	Feeding regime	1	67	88.82	<0.001
	Total no. of matings	3	67	0.99	0.404

ndf/ddf: numerator and denominator degrees of freedom.

reproductive output among small males. We discuss each of these four points below.

Total number of matings

Small nonfed males mated on average less often than well-fed or large males (Fig. 1). This pattern is more likely to be driven by longer refractory periods for nonfed small males than by female choice, as females invariably courted all these males. The production of nutritious ejaculates carries costs in terms of acquiring food resources and replenishing an ejaculate takes time, especially when food resources are scarce (Gwynne, 1993; Perry & Tse, 2013; Svärd & Wiklund, 1989). Much like females, which show a refractory period after mating to process an ejaculate (Parker, 1998 ; Simmons & Gwynne, 1991; Wedell, 1993), male *M. dorsalis* show a refractory period during which their ejaculate is replenished (Salehialavi et al., 2011) and this period might be prolonged in nonfed small males. In ladybird beetles, low-condition males consume more food after matings with spermatophore transfer and mate less often than high-condition males, suggesting that ejaculate production requires both food resources and time (Perry & Tse, 2013).

Ejaculate size

Ejaculate size and composition affect male reproduction in several ways, including nutritional contributions to female fecundity, physiological manipulation of female postmating behaviour and success in sperm competition (Blay & Yuval, 1997; Vahed, 1998). In species in which females are able to digest ejaculates, male fitness may depend on the amount of resources provided, provided that females use these resources to produce eggs that are fertilized by the provisioning male's sperm (Greeff & Michiels, 1999). Our results that well-fed males transferred heavier ejaculates during mating (Fig. 1) and produced more offspring than nonfed males may seem consistent with this general scenario. However, larger ejaculates suppress female remating more efficiently (Arnqvist & Rowe, 2005) and can also otherwise increase male sperm competition success (Parker, 1998; Parker & Ball, 2005; Simmons, 2001). In fact, a recent study showed that selection via sperm competition, rather than through resource provisioning to mates, is primarily responsible for the maintenance of large male ejaculates in *Megabruchidius* (Booksmythe, Fritzsche, & Arnqvist, 2014).

Ejaculates can be costly to produce (Dewsbury, 1982), especially in species in which males transfer nutritious substances that confer direct benefits to females (Gwynne, 1988; Moya-Larano & Fox, 2006; Svärd & Wiklund, 1989). Because the transfer of large nutritious ejaculates is costly and depends on food availability in *M. dorsalis*, males might allocate their ejaculate strategically to maximize their fitness if females vary in reproductive quality (Galvani & Johnstone, 1998; Reinhold, Kurtz, & Engqvist, 2002; Rubolini et al., 2006; Wedell, Gage, & Parker, 2002). Both well-fed and nonfed males showed reduced ejaculate allocation over the course of three matings. It is unclear what exactly caused this pattern, but one possible explanation might be cryptic male choice for virgin and/or novel females (Dewsbury, 1981; Reinhold et al., 2002). Males of several insects are known to be capable of sensing a female's mating status and to tailor their ejaculate expenditure based on this information (Delbarco-Trillo, 2011; Engqvist & Reinhold, 2006; Kelly & Jennions, 2011; Parker, 1998; Parker & Ball, 2005).

Reproductive output

Male condition had large direct effects on male (and female) reproductive productivity, manifested through effects of both male body size and male feeding regime (Fig. 3b). The fact that females that were mated to small and/or nonfed males produced fewer
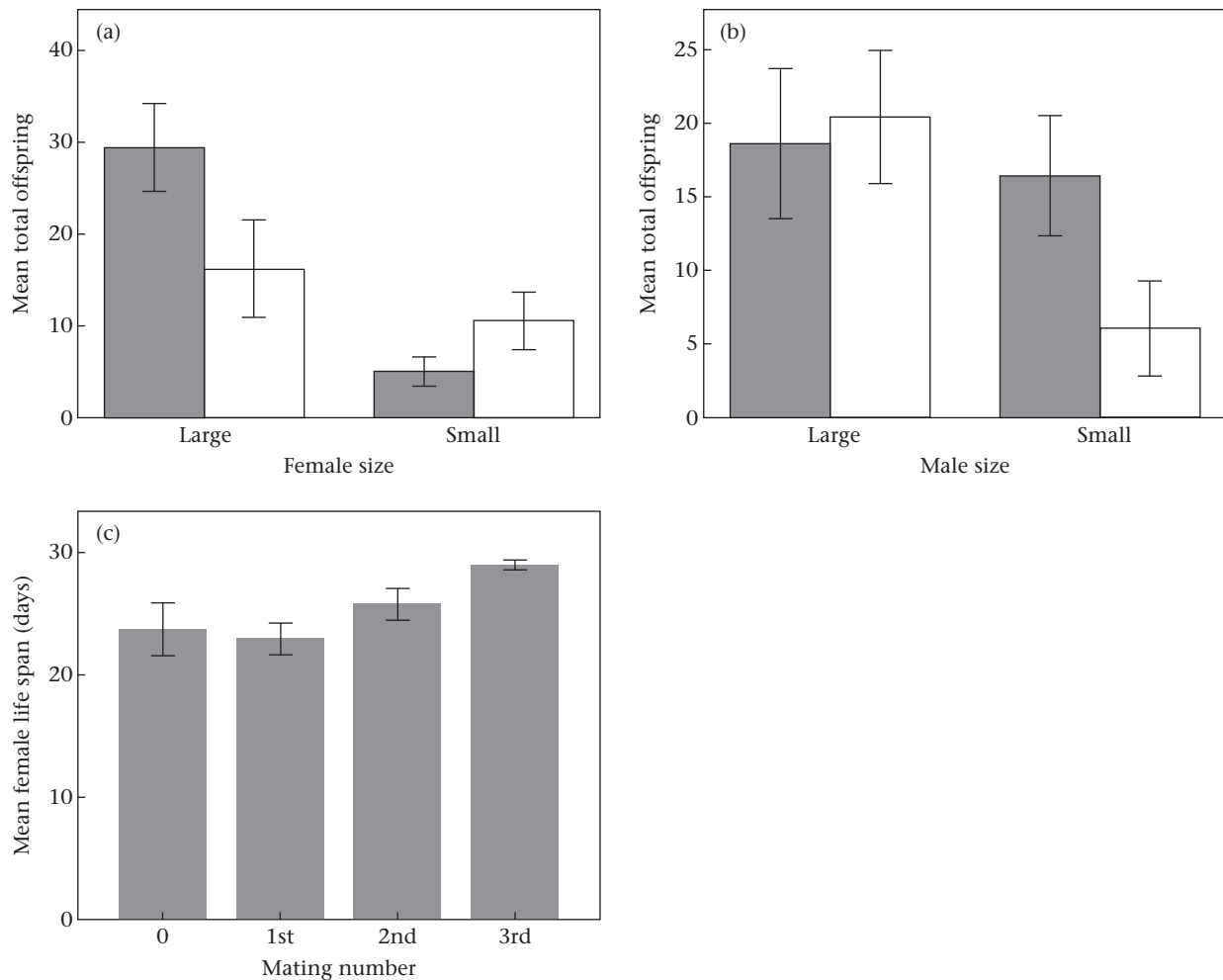
Figure 3. Mean ± SE fitness components. (a) Total number of offspring of large and small females in relation to whether they were fed (grey bar) or nonfed (white bar). (b) Total number of offspring of large and small males in relation to whether they were fed (grey bar) or nonfed (white bar). (c) Life span of females in relation to the number of times they mated.

offspring is consistent with a lower nutritional value in these males' ejaculates. Interestingly, females mated to nonfed males lived for longer than those mated to well-fed males. This is probably due to the higher reproductive rate of the latter females.

Since males that produce large ejaculates have higher post-mating reproductive success (Takakura, 1999), ejaculate size is under cryptic female choice. When mating with males that produce larger or more nutritious ejaculates, females might be more likely to allocate these ejaculates towards offspring production (Partridge, Green, & Fowler, 1987; Rooney, 1999; Takakura, 2004; Wedell & Karlsson, 2003) rather than somatic maintenance. However, large and well-fed males might also be able to produce proteins that manipulate resource allocation in females and thus bias allocation towards offspring production. It is very difficult to disentangle these possibilities (Eberhard, 1996; Sirot, Wolfner, & Wigby, 2011). We note that variation in ejaculate composition, in addition to size, probably contributes to variation in male and female reproductive success in *Megabruchidius*.

Life span

The costs of mating have been relatively well studied in females (Arnqvist & Rowe, 2005), but studies on mating costs for males are still scarce (Perry & Tse, 2013; Perry, Sirot, & Wigby, 2013; Wedell et al., 2002). We did not observe a significantly shorter life span

in males that mated more often, which was seen in a previous study (Salehialavi et al., 2011). We suggest that three matings is insufficient to generate sizeable costs in terms of life span, especially given that individuals mate many times under natural conditions (Takakura, 2006). Overall, resource availability was the strongest predictor of life span. Males with access to adult food resources lived significantly longer than males without food, by far outweighing positive effects of large body size on life span (Table 3).

We have shown that the amount of nutrition provided in the ejaculate is related to both male body size and male reproductive output. Our results also suggest that male body size is under both pre- and postcopulatory sexual selection, with larger body size being associated with higher reproductive productivity regardless of food availability (Fig. 3b). The ability to provide large nutritious ejaculates may consequently generate positive selection on male body size that is analogous to fecundity selection in females (Fedorka & Mousseau, 2002b; Jia et al., 2000). This might contribute to the fact that male *M. dorsalis* are larger than females, whereas other seed beetles show the opposite pattern (Fox, Stillwell, & Moya-Laraño, 2007). We note that body size variation in our experiment reflects variance in juvenile resource acquisition that is due to both differences in acquisition efficiency, which may in part have a genetic basis, and environmental differences in resource quantity and quality. Given the major environmental

effects on body size in seed beetles (Fedorka & Mousseau, 2002b) and the fact that the resources provided in our experiment (i.e. beans) varied markedly in size and quality, we suggest that environmental factors dominated in our experiment.

The Female Perspective: Size and Direct Benefits

We found that (1) large females produced more offspring than small females and (2) multiple mating increased the life span of all females. Again, we discuss each of these points below.

Reproductive output

Large females with access to well-fed males showed the highest reproductive productivity and females that mated multiply produced significantly more offspring (Fig. 3a), in line with previous studies (Arnqvist & Nilsson, 2000; Fedorka, & Mousseau, 2002a,b; Jennions & Petrie, 2000). Although mating with well-fed males increased fecundity for large females, it did not do so for small females. One possible explanation is that smaller females allocate a larger fraction of the ejaculate resources to somatic maintenance, rather than to reproduction. This might be because small females are incapable of increasing their reproductive output due to physiological constraints (Honĕk, 1993).

Life span

Females that mated multiply lived longer (Fig. 3c) and had overall higher reproductive output, in line with previous studies (Arnqvist & Nilsson, 2000; Fedorka, & Mousseau, 2002a,b; Jennions & Petrie, 2000). However, taking female size into account, small females showed an increase in life span but not in offspring production. Again, large and small females may allocate ejaculate resources differently.

Phenotypic Condition and the Honeylocust Beetle Mating System

We have shown that the phenotypic condition of males and females had sizeable independent and interactive effects on mating and reproductive productivity in this role-reversed species. In males, body size had significant but relatively marginal effects on male mating and reproductive output. Male adult feeding, in contrast, had sizeable effects on almost all aspects of male and female reproduction, through the nutritional effects of ejaculates in females. Considering these facts, it is not surprising that both males and females show premating preferences for large mates and that males show mating preferences for female traits that correlate with phenotypic condition (Salehialavi et al., 2011). Further, the fact that the mating system shows pronounced female polyandry and sex role reversal in courtship is predicted given the direct resource-based mating interactions. Moreover, the ecology and life histories of males and females have diverged: as is the case in some other sex role-reversed insect mating systems, such as dance flies (Svensson, 1997) and certain butterflies (Wiklund, Kaitala, & Wedell, 1997), male honeylocust beetles are mobile and forage for food resources while females are more resident at the host plant where they essentially forage for matings (Takakura, 2004). The fact that large ejaculates are favoured in males by both sperm competition (Takakura, 1999), premating sexual selection (Salehialavi et al., 2011) and fecundity selection (shown here) results in the strong net selection on male body size observed in *Megabruchidius* (Fritzsche & Arnqvist, 2013) which, no doubt, contributes to the unusual male-biased sexual size dimorphism seen in this genus compared to other seed beetles (Fox et al., 2007).

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