

Sperm competition generates evolution of increased paternal investment in a sex role-reversed seed beetle

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Abstract

When males provide females with resources at mating, they can become the limiting sex in reproduction, in extreme cases leading to the reversal of typical courtship roles. The evolution of male provisioning is thought to be driven by male reproductive competition and selection for female fecundity enhancement. We used experimental evolution under male- or female-biased sex ratios and limited or unlimited food regimes to investigate the relative roles of these routes to male provisioning in a sex role-reversed beetle, *Megabruchidius tonkineus*, where males provide females with nutritious ejaculates. Males evolving under male-biased sex ratios transferred larger ejaculates than did males from female-biased populations, demonstrating a sizeable role for reproductive competition in the evolution of male provisioning. Although larger ejaculates elevated female lifetime offspring production, we found little evidence of selection for larger ejaculates via fecundity enhancement: males evolving under resource-limited and unlimited conditions did not differ in mean ejaculate size. Resource limitation did, however, affect the evolution of conditional ejaculate allocation. Our results suggest that the resource provisioning that underpins sex role reversal in this system is the result of male–male reproductive competition rather than of direct selection for males to enhance female fecundity.

Introduction

In some insects, the sex roles are reversed in the sense that females actively court males, whereas males are choosy and often reluctant to mate. Complete or partial role reversal occurs, for example, in certain crickets (Gwynne, 1981, 1993; Simmons, 1992), beetles (Takamura, 1999; Salehialavi *et al.*, 2011), butterflies (Leimar *et al.*, 1994; Jiggins *et al.*, 2000), barklice (Wearing-Wilde, 1996), water bugs (Smith, 1979) and flies (Funk & Tallamy, 2000). Theory suggests that role reversal should occur when males evolve to provide resources to females at mating, such as large ejaculates, spermatophores, prey items or oviposition substrates (Smith, 1979; Gwynne & Simmons, 1990; Leimar *et al.*, 1994;

Wearing-Wilde, 1996). Because these resources are costly to produce or otherwise limited, male potential reproductive rate is lowered. In more extreme cases, this may render the operational sex ratio female-biased, and the Bateman gradient steep in females relative to males (Simmons & Bailey, 1990; Robson & Gwynne, 2010; Fritzsche & Arnqvist, 2013). This selects for sex role reversal in courtship (Clutton-Brock & Vincent, 1991; Clutton-Brock & Parker, 1992; Parker & Simmons, 1996). Two distinct, but not mutually exclusive, forms of sex-limited selection in males may cause role reversal by selecting for increased provisioning of resources to females (Vahed, 1998). First, males can benefit from enhancing the fecundity of their mate by providing scarce resources that constrain female reproductive output (Thornhill, 1976; Boggs, 1990; Tallamy, 1994). Second, males that provide resources to females often enjoy increased mating or fertilization success in the face of male–male reproductive competition (Thornhill, 1976; Sakaluk, 1984; Wedell, 1991). Selection via reproductive competition is thought to be the stronger force in the evolution of nuptial gifts. However

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it is striking that wherever male provisioning has led to sex role reversal, selection via fecundity enhancement has typically been assumed to be the driver because the direct positive effect of resources for females is what is causing mating system evolution (Vahed, 1998). This is potentially misleading because both routes to the evolution of male provisioning will in effect constitute paternal investment (Zeh & Smith, 1985; Quinn & Sakaluk, 1986; Gwynne, 1991) and both may therefore cause the evolution of sex role reversal. Although previous work has shown that males in role-reversed insects plastically adjust their provisioning to females in response to both resource availability and the level of mating competition (Gwynne & Simmons, 1990; Simmons & Bailey, 1990; Simmons & Kvarnemo, 1997), no study has experimentally evaluated the relative roles of fecundity enhancement and reproductive competition in the evolution of male provisioning in a role-reversed system.

The most common form of resource provisioning by male insects is the transfer of large ejaculates to females at mating (Vahed, 1998). In addition to sperm, ejaculates often include water, nonorganic substances and a wide range of organic compounds, such as peptides and proteins (Arnqvist & Rowe, 2005; Poiani, 2006; Perry *et al.*, 2013), many of which are metabolized and used by females as direct resources for reproduction. Large ejaculates are associated both with an increase in the number or condition of offspring produced from a mating (Wedell, 1996; Moya-Laraño & Fox, 2006) and with increased fertilization success through delaying female remating or otherwise increasing a male's paternity share under sperm competition (Eady, 1995; Katvala *et al.*, 2008; McNamara *et al.*, 2009; Parker & Pizzari, 2010). Ejaculates are costly (Dewsbury, 1982), and gains from increased investment in a single mating encounter must be weighed against gains from investment in future mating partners (Wedell *et al.*, 2002). In terms of fecundity enhancement, females (and thus also males) stand to benefit most from male provisioning when food resources are scarce (Simmons & Bailey, 1990). Thus, selection on males to enhance female fecundity should be elevated under conditions of food limitation, resulting in the evolution of larger ejaculates (Markow *et al.*, 1990). In terms of reproductive competition, the degree of polyandry and the level of competition among males for fertilizations are known to strongly influence the evolution of ejaculate traits (Pitnick *et al.*, 2001; Ingleby *et al.*, 2010). The relative importance of pre- and post-copulatory competition to male fitness, and the extent to which there is a trade-off between these two components of reproductive effort, shapes the overall investment in ejaculates (Parker *et al.*, 2013; Lüpold *et al.*, 2014). In general, a higher risk of sperm competition is predicted to result in the evolution of increased ejaculate allocation (Parker *et al.*, 1997; Parker & Pizzari, 2010). Across species,

positive relationships between the degree of sperm competition and measures of ejaculate investment support this prediction (see table 1 in Parker *et al.*, 1997).

Here, we used an experimental evolution approach to assess the relative roles of fecundity enhancement and male–male reproductive competition in the evolution of direct resource provisioning by males, in a seed beetle system where such resources have caused sex role reversal. This has proven difficult using comparative data because the effects of polyandry on the evolution and maintenance of male provisioning are complicated by coevolutionary feedback between male investment and the mating rates of both sexes (Williams *et al.*, 2005; Vahed & Parker, 2011). For example, ejaculates conferring direct benefits to females can promote increased female mating rates and polyandry, in turn increasing sperm competition (Vahed, 2006; Rönn *et al.*, 2008). An increase in female mating rate entails an increase in male mating rate, which predicts reduced ejaculate allocation per mating (Parker & Ball, 2005; Katvala *et al.*, 2008). Importantly, this will only be true when sex ratios are equal. Experimental evolution of populations under male-biased and female-biased sex ratios provides a powerful technique to manipulate male and female mating rates independently.

In the honey locust beetle *Megabruchidius tonkineus* Pic (Bruchidae), as in many seed beetles, males transfer a very large ejaculate representing up to ~7% of their body mass to females (Takakura, 1999). Unlike other seed beetles, however, females in this genus gain dramatic fecundity benefits from mating: the number of offspring a female produces increases by about 10 offspring (~20% of the average singly mated female's reproductive output) with each additional mating (Takakura, 1999, 2006; Fritzsche & Arnqvist, 2013). Matings impose survival costs on males (Salehialavi *et al.*, 2011), and both sexes show refractory behaviour for many hours after mating (Takakura, 2001). The duration of the female refractory period is positively related to ejaculate size (Takakura, 2001) and females process ejaculate resources at a higher rate than males can produce them (Takakura, 2004). Female mating propensity increases as resource availability decreases, and they increase feeding behaviour when mated to poor-condition males (Takakura, 2004) reflecting the nutritional role of the ejaculate. The sex roles in courtship are reversed in honey locust beetles compared to other seed beetles: females actively initiate matings, court males and have exaggerated secondary sexual traits that they employ during courtship, whereas males often reject female mating attempts (Takakura, 1999, 2001; Salehialavi *et al.*, 2011).

We used a crossed experimental evolution design where replicated populations evolved under different adult sex ratios and food regimes to examine the evolution of male resource provisioning to females. If male resource provisioning is the result of selection for

fecundity enhancement, we predict the evolution of larger ejaculates under food limitation. If resource provisioning is instead the result of male–male reproductive competition, we predict the evolution of larger ejaculates under male-biased sex ratios.

Materials and methods

Experimental evolution

The lines studied here derive from a large (> 500 individuals) laboratory stock population originally seeded by a sample of > 300 field-collected adult *M. tonkineus* (Orczy-kert, Budapest, Hungary; May 2009). All beetles were maintained in climate-controlled chambers at 26 °C and 70 ± 10% relative humidity, on a 16:8 h light:dark cycle. The experimental lines were allowed to evolve in the laboratory for 19 generations, using a factorial 2 × 2 design with four replicates of each cell ($N = 16$ lines in total). Lines were kept either at male-biased (125M : 25F) or female-biased (25M : 125F) sex ratio conditions and beetles were either fed (with sugar-water and pollen) or unfed. Under male-biased conditions, male mating rate will be relatively low, female mating rate will be high, and reproductive competition between males will be intense. Food availability has profound effects in honey locust beetles, affecting for example lifespan, the cost of reproduction and male ejaculate size (Takakura, 2001, 2004, 2006; Salehialavi *et al.*, 2011).

Every generation, 150 virgin adults per line were placed at the desired sex ratio in clean 1 L glass jars containing a breeding substrate of beans of the honey locust *Gleditsia triacanthos*. Adults were allowed to mate and lay eggs in these jars for ~2 weeks and were then removed and discarded. Females cement their eggs to the sides and lid of the jar; the larvae hatch after 5–7 days and bore into a bean to complete their development (3–4 weeks). A pilot experiment indicated that females with access to food produced substantially more eggs, and we used these results to estimate the amount of beans required to keep larval competition constant across treatments: ‘fed’ lines were provided with 375 g beans and ‘unfed’ lines with 120 g beans. To keep the substrate volume and structural complexity in the jars constant across food treatments, each jar was filled partly with ‘dummy beans’ (LECA clay balls; ~20 g for ‘fed’ and ~100 g for ‘unfed’ treatments). ‘Fed’ lines were provided with 20% sucrose solution, an ample supply of pollen granules (Bee Pollen Capsules; Manuka Health Ltd., Auckland, New Zealand) and distilled water. ‘Unfed’ lines were provided with only distilled water.

During the larval development period, before the emergence of new beetles from the beans, we distributed beans from each line individually in single wells of 24-well culture plates to allow the collection of vir-

gin adults for the next generation. We note that, unlike in seed beetles with conventional sex roles, sex-specific variances in reproductive fitness are statistically indistinguishable in *M. tonkineus* (Fritzsche & Arnqvist, 2013). Thus, the two sex ratio treatments used here should show similar effective population sizes ($N_e \sim 83$).

The experimental conditions were terminated at generation 20, and lines were maintained under common garden conditions of equal sex ratios without access to food or water for four subsequent generations. This ensured that the results of our assays were not affected by phenotypic plasticity due to differing maternal and paternal sex ratio and food conditions. Due to an infection of our lines with an ectoparasitic mite (*Pyemotes* sp.), five of the 16 lines were lost during the transfer to common garden conditions, leaving two replicate lines of the ‘female-biased × fed’ treatment and three replicates in each of the other three cells in our design. Some of the surviving lines experienced greater reductions in population size than others. In four lines, the number of individuals founding the 21st generation was relatively low (25–50 individuals), whereas the number of founders was higher (> 70 individuals) in the other 7 lines. We thus created a binary ‘mite score’ to test for potential effects of this population size reduction in our analyses.

Cross-population mating design

Virgin adults from generation 24 were collected from each of the 11 experimental evolution lines. We conducted matings within and between all four treatments following as closely as possible a fully crossed design. For crosses within treatments, mating pairs never originated from the same line to avoid confounding treatment effects with line-specific effects. We ensured that, to the extent it was possible, every line was crossed with every other line and that each cross was replicated using both sexes from both lines involved (i.e. crosses between lines A and B would include matings of A males with B females and B males with A females).

Virgin adults were collected and paired on the 10th day following emergence. We used a Sartorius® ME/SE analytical microbalance (Sartorius AG, Goettingen, Germany) to weigh every individual to the nearest 0.1 mg before mating. Pairs were placed together in 3-cm-diameter plastic petri dishes and observed until they had mated once. Successful intromission can be recognized by a characteristic backwards-leaning posture of the mounted male and almost complete lack of motion by either partner for the 2–7 min duration. We counted this as a mating, although not every mating results in successful sperm transfer. Pairs that did not mate within 3 h were separated overnight and placed together again the following day. Pairs that did not mate on their second encounter were discarded. After mating, pairs were

separated, and each individual was weighed again. The difference in an individual's weight before and after mating provides an estimate of ejaculate weight (Edvardsson & Tregenza, 2005). Males were then discarded, and females were placed individually in 12-cm-diameter glass petri dishes containing 100 g *G. triacanthos* beans and checked daily until death to record lifespan. We recorded the number of offspring that had emerged in each dish after 9 weeks to provide a measure of female lifetime reproductive success.

Statistical analysis

We used linear mixed models fitted by restricted maximum likelihood to examine the effects of the sex ratio and food treatments on ejaculate weight and female post-mating performance. In these models, male and female line IDs were always included as random effect factors to account for any line-specific effects. Line ID effects were always low and never statistically significant (95% CI of variance components enclosed zero in all cases) and are thus not detailed further here. In our inferential model of ejaculate weight, we included the sex ratio of the male line and the food treatment of the male line as categorical fixed effect factors, and initial male weight and initial female weight as continuous covariates. In the inferential model of female lifespan, we included sex ratio of the female line, food treatment of the female line, initial female weight, ejaculate weight and total offspring produced.

To test for effects of sex ratio and food treatment on the number of offspring produced from a single mating, we fitted a generalized linear mixed model with a Poisson error distribution and log link function and an empirically derived dispersion parameter to control for overdispersion. Here, we included the unique line ID combination (male line by female line) as a random effects factor to account for any line-specific effects. Sex ratio of the male line, sex ratio of the female line, food treatment of the male line and food treatment of the female line were included as factorial variables, and initial female weight and ejaculate weight were included as continuous predictor variables. Because our data contained females that produced no offspring, we assessed whether this model appropriately controlled for overdispersion by fitting an analogue generalized linear mixed model of whether females produced any offspring or not (i.e. 0 or 1) with a binomial error distribution and logit link function.

We initially included all two-way interactions between predictor variables and then sequentially excluded nonsignificant interaction terms. We note that in all presented models, statistically significant effects remained consistent across the model reduction process. For all excluded terms, we present the parameter estimate taken from the model immediately prior to their exclusion in Tables S1–S3. We

also note that the inclusion of the 'mite scores' of the male and female line as fixed effect factors did not in any case affect the qualitative outcome of any of the models, and we thus chose to exclude these two terms from our final inferential models to avoid overparameterization. Models were fitted in JMP v.10 (SAS Institute, Cary, NC, USA) and GenStat v.10.2 (VSN International Ltd., Hemel Hempstead, UK).

Results

One hundred and twenty four of 157 pairs successfully mated during their first encounter, with a further 20 pairs mating on the second day (final $n = 144$). Male and female body weights were uncorrelated (mean \pm SD males = 21.9 ± 2.4 mg, females = 17.9 ± 2.1 mg, $r = 0.005, P = 0.95$). Male weight was not affected by selection history (ANOVA: sex ratio treatment, estimate \pm SE = 0.373 ± 0.197 , $F_{1,140} = 3.57$, $P = 0.06$; food treatment, estimate \pm SE = -0.044 ± 0.197 , $F_{1,140} = 0.05$, $P = 0.82$; sex ratio \times food treatment, estimate \pm SE = -0.036 ± 0.197 , $F_{1,140} = 0.03$, $P = 0.86$). Although sex ratio treatment and food treatment did not affect female weight (sex ratio treatment, estimate \pm SE = -0.026 ± 0.174 , $F_{1,140} = 0.02$, $P = 0.88$; food treatment, estimate \pm SE = -0.083 ± 0.174 , $F_{1,140} = 0.23$, $P = 0.63$), there was a significant effect of their interaction (estimate \pm SE = -0.615 ± 0.174 , $F_{1,140} = 12.54$, $P < 0.001$), such that females evolved a larger size in lines experiencing the 'male-biased \times unfed' and 'female-biased \times fed' treatment combinations. Line means for male and female body size, as well as our three main variables of interest (ejaculate size, number of offspring produced and female lifespan) are presented in Tables S4–S6.

Ejaculate weight

The change in male weight during copulation was significantly correlated with the change in female weight ($r = 0.569$, $P < 0.0001$). The fact that the correlation was not stronger (Edvardsson & Tregenza, 2005) is likely due to partial ejaculate dumping and oral consumption immediately after copulation by some females, a phenomenon common in insects (Perry & Rowe, 2008) and observed by us in *M. tonkineus*. In support of this interpretation, males lost slightly more weight on average than females gained during mating (see Savalli & Fox, 1998; for a very similar result and interpretation in another seed beetle species). We therefore chose to use male weight loss as a measure of ejaculate size, as is standard in studies of seed beetles (Savalli & Fox, 1998; Rönn *et al.*, 2008).

The sex ratio experienced by the male line significantly affected the weight of the ejaculate. Males from male-biased lines transferred significantly larger ejaculates during mating than did males from female-biased

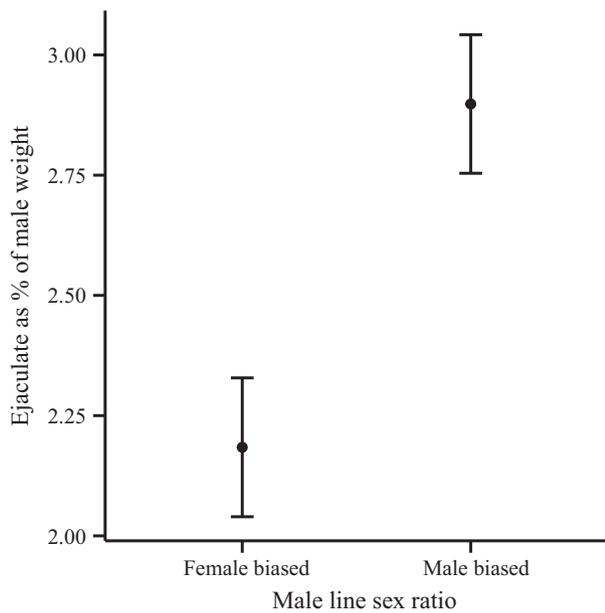


Fig. 1 Mean (\pm SE) percentage weight of the ejaculate transferred during a single mating by males from experimental evolution lines with female-biased and male-biased sex ratios. Males from male-biased lines evolved to transfer considerably larger ejaculates.

lines (Fig. 1, Table 1). This was the case whether we used absolute ejaculate weight, or ejaculate weight relative to male body weight in our analyses (in fact, the models were nearly identical). Thus, lines experiencing intensified male–male reproductive competition evolved a larger body mass-specific ejaculate size. Neither the food treatment of the male line nor the weights of either sex were significant as main effects. However, there was a significant interaction between male food treatment and female weight (Fig. 2, Table 1). Males from ancestrally fed populations transferred heavier ejaculates to heavier females, whereas males from unfed populations did not. In a separate model, we also tested for effects of female selection history, but neither the sex ratio treatment nor food treatment of the female line, or their interactions with each other or with male selection history affected ejaculate weight (all $P > 0.05$).

Number of offspring produced

The number of offspring produced increased with female weight and with the weight of the ejaculate transferred by the male. There was, however, no effect of the sex ratio or food treatment of either the male or female line on the number of offspring produced, when controlling for variation in female weight and ejaculate size (Table 2). An analogue model, based on a binomial error distribution, largely validated these results. Here, male ejaculate size also significantly affected offspring production ($\chi^2_1 = 4.87$, $P = 0.027$), whereas female body weight no longer had a significant effect ($\chi^2_1 = 2.54$, $P = 0.111$). All other factors had clearly nonsignificant effects on offspring production (all $P > 0.612$).

Female lifespan

Female lifespan was negatively related to reproductive output, such that females that produced more offspring died younger (Table 3). Lifespan increased with initial female weight, but was not significantly affected by either the sex ratio or food treatment of the female line, or by ejaculate weight (Table 3). Thus, although females benefited from receiving larger ejaculates in terms of increased reproductive output, these greater resources did not allow females to escape the costs of reproduction. A separate model indicated that neither male selection history nor its interaction with female selection history affected female lifespan (all $P > 0.05$).

Discussion

Male ejaculates evolved to a larger size under conditions of intense male–male competition. In contrast, the fecundity-enhancing effects of provisioning females with ejaculate resources had no apparent effect on the evolution of ejaculate size, as ejaculate size did not differ between lines experiencing conditions of food limitation and abundance. Ejaculate size was positively related to female offspring production, confirming previous results that showed that the ejaculate resources provided by males are indeed key to female fitness in this species (Takakura, 1999, 2006; Fritzsche & Arnqvist, 2013). Thus, males evolved a higher degree of

Table 1 Tests of fixed effects (type III) on ejaculate weight relative to male body weight.

	Estimate	SE	F	P
Intercept	2.8421	1.3402		
Male line sex ratio	−0.3155	0.1023	9.501 _{1,136.3}	0.003
Male line food treatment	0.1470	0.1004	2.144	0.145
Male weight	−0.0628	0.0440	2.041	0.155
Female weight	0.0608	0.0506	1.445	0.231
Male line food treatment × female weight	−0.1210	0.0500	5.855 _{1,136.7}	0.017

Values in bold are significant at $\alpha = 0.05$.

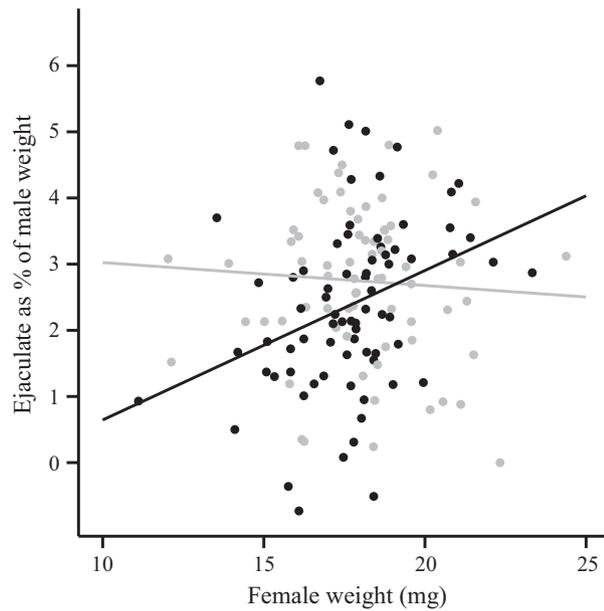


Fig. 2 The relationship between female weight and the weight of ejaculate (as a percentage of male body weight) transferred by males from food limited (grey) and unlimited (black) experimental evolution lines. Males from unlimited lines transferred larger ejaculates to larger females, whereas males from food limited lines did not.

Table 2 Tests of fixed effects (type III) on lifetime offspring production of females following a single mating.

	Estimate	SE	F	P
Intercept	2.9530	0.3158		
Male line sex ratio	-0.3339	0.2820	0.10 _{1,46}	0.750
Female line sex ratio	-0.3581	0.2769	1.48 _{1,41.6}	0.230
Male line food treatment	0.0615	0.2775	0.05 _{1,38.5}	0.829
Female line food treatment	0.16672	0.2753	0.24 _{1,41}	0.626
Female weight	0.1438	0.0581	7.32 _{1,136.4}	0.008
Ejaculate weight	1.0010	0.4324	5.36 _{1,132.2}	0.022

Values in bold are significant at $\alpha = 0.05$.

paternal investment under conditions of intense male-male competition, but not under conditions where such investment is of higher direct value to females.

Our results have important implications for the evolution and maintenance of sex role reversal, because the ejaculate resources provided by males underpin role reversal in this system (Takakura, 1999). Males that evolved under resource-limited conditions, where selection for female fecundity enhancement was elevated, did not evolve to provide more direct resources to females. However, males that evolved under conditions of elevated male-male competition did evolve to provide more direct resources to females. This suggests that male provisioning, at the centre of role reversal in honey locust beetles, is more strongly driven by the indirect effects of male-male reproductive competition than by direct selection for female fecundity enhancement.

Our findings parallel those in several insects with conventional sex roles, where increased male-male competition leads to the evolution of increased investment in ejaculates. Imposing monandrous mating regimes on naturally polyandrous dung flies, fruit flies, dung beetles and seed beetles removed male mating competition, resulting in the evolution of reduced testis size (a common proxy for ejaculate production) (Hosken & Ward, 2001; Pitnick *et al.*, 2001; Simmons & Garcia-Gonzalez, 2008; Gay *et al.*, 2009), whereas in *Drosophila pseudoobscura*, the evolutionary response of males to increased mating competition was the enlargement of the accessory glands (Crudginton *et al.*, 2009). In contrast, Wigby & Chapman (2004) found no difference in testis or accessory gland size of males from male- and female-biased populations after 32 generations of experimental evolution in *Drosophila melanogaster*. However, ejaculate production is also expected to increase when male mating rate is elevated (Vahed & Parker, 2011), and testis size increased in males evolving under female-biased conditions in *D. melanogaster* (Reuter *et al.*, 2008). Our approach allowed us to directly measure evolved differences in the ejaculate invested per mating and separate the effects of elevated male mating rate (female-biased sex ratios) and increased male-male reproductive competition (male-biased sex ratios). The only other study to have done this found similar support for the role of male reproductive competition in ejaculate evolution: in the moth *Plodia interpunctella*, males experimentally evolving

Table 3 Tests of fixed effects (type III) on female lifespan (days) following a single mating.

	Estimate	SE	F	P
Intercept	4.3622	3.0698		
Female line sex ratio	0.5046	0.6201	0.662 _{1,7.4}	0.441
Female line food treatment	0.4302	0.6211	0.480 _{1,7.2}	0.510
Female weight	1.5249	0.1700	80.486 _{1,137.7}	< 0.0001
Ejaculate weight	-0.4007	1.2618	0.101 _{1,135.4}	0.751
Number of offspring	-0.0865	0.0169	26.062 _{1,134.1}	< 0.0001

Values in bold are significant at $\alpha = 0.05$.

under male-biased sex ratios transferred more sperm to females than did males from female-biased lines (Ingley *et al.*, 2010).

It has previously been suggested that selection on male resource provisioning through fecundity enhancement should often be a relatively weak force in insects (Vahed, 1998). This prediction derives from several facts. Because last male sperm precedence is high and incorporation of ejaculate resources into offspring often takes longer than the female remating interval, provisioning males may often find themselves primarily investing in offspring that are fathered by subsequent males (Wickler, 1985, 1986; Gwynne, 1986; Simmons & Parker, 1989; but see Sakaluk, 1986). Additionally, the marginal value of an increase in resource provisioning may be low because of diminishing rewards to females with increasing ejaculate size (Parker & Simmons, 1989). Selection via reproductive competition may of course result in male contribution to offspring production, such that male provisioning is an incidental effect of reproductive competition (Quinn & Sakaluk, 1986; Eberhard, 1996; Arnqvist & Rowe, 2005). Indeed, materials of male origin have been identified in eggs and/or offspring of several insect species (Huignard, 1983; Pitnick *et al.*, 1991; Gwynne & Brown, 1994). It has also been suggested that selection via fecundity enhancement is more likely to contribute to the maintenance, rather than the origin, of ejaculate provisioning (Simmons & Parker, 1989), although we found no support for this here. For example, reducing the risk of sperm competition by inducing a longer refractory period should increase a male's certainty/share of paternity, thus increasing the potential benefits to the male of investment into offspring (Zeh & Smith, 1985; Quinn & Sakaluk, 1986).

The transfer of larger ejaculates to larger females is common in insects, where female size often correlates with fecundity (Wedell *et al.*, 2002; Kelly & Jennions, 2011). Such conditional allocation increases the returns on male investment and/or the risk of sperm competition, both scenarios predicting the transfer of larger ejaculates (Gage, 1998; Wedell & Cook, 1999; Wedell *et al.*, 2002). We found that males from 'fed', but not 'unfed', lines showed greater ejaculate allocation when mated to larger females. This represents an evolved difference in allocation strategy, as all individuals used in the mating trials were raised under identical conditions. While intuitively, allocation strategies should be most important under resource-limited conditions (Engqvist & Sauer, 2001), here conditional allocation was lost under food limitation. We see two scenarios that might explain the observed pattern. First, males may need a certain minimum ejaculate size to mate, such that differential allocation is only possible when males can acquire additional resources. In this case, 'unfed' line males may have lost the ability to differentially allocate as extra resources were not available. Second, com-

pared to the treatment conditions under which the populations evolved, the common garden conditions of no food or water entailed a relatively greater decrease in resource availability for 'fed' lines than for 'unfed' lines. 'Fed' line males may thus have experienced these conditions as an unusually harsh environment, requiring prudent allocation. Although conditional ejaculate allocation is widely expected and frequently shown to occur, the factors favouring its evolution have been largely neglected. Our somewhat counterintuitive result suggests that this could be an illuminating avenue for future research.

Here, we have shown that increased male-male reproductive competition strongly selects for larger male ejaculates in a sex role-reversed seed beetle. As females gain fecundity benefits from ejaculate resources in this system, male-male reproductive competition results in indirect selection for paternal investment. In contrast, resource limitation had little effect on the evolution of ejaculate size in our experimental populations, despite increased direct selection on fecundity enhancement benefits. Our results support the tenet that role reversal in courtship can be the indirect result of male-male competition in taxa where male success in intra-sexual competition is directly or indirectly related to investment in mate provisioning.

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References

- Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton University Press, Princeton, NJ.
- Boggs, C.L. 1990. A general model of the role of male-donated nutrients in female insects' reproduction. *Am. Nat.* **136**: 598–617.
- Clutton-Brock, T.H. & Parker, G.A. 1992. Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* **67**: 437–456.
- Clutton-Brock, T.H. & Vincent, A.C.J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature* **351**: 58–60.
- Crudginton, H.S., Fellows, S., Badcock, N.S. & Snook, R.R. 2009. Experimental manipulation of sexual selection promotes greater male mating capacity but does not alter sperm investment. *Evolution* **63**: 926–938.
- Dewsbury, D.A. 1982. Ejaculate cost and male choice. *Am. Nat.* **119**: 601–610.

- Eady, P.E. 1995. Why do male *Callosobruchus maculatus* beetles inseminate so many sperm? *Behav. Ecol. Sociobiol.* **36**: 25–32.
- Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, NJ.
- Edvardsson, M. & Tregenza, T. 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behav. Ecol.* **16**: 788–793.
- Engqvist, L. & Sauer, K.P. 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. *Proc. R. Soc. B* **268**: 729–735.
- Fritzsche, K. & Arnqvist, G. 2013. Homage to Bateman: sex roles predict sex differences in sexual selection. *Evolution* **67**: 1926–1936.
- Funk, D.H. & Tallamy, D.W. 2000. Courtship role reversal and deceptive signals in the long-tailed dance fly, *Rhamphomyia longicauda*. *Anim. Behav.* **59**: 411–421.
- Gage, M.J.G. 1998. Influences of sex, size, and symmetry on ejaculate expenditure in a moth. *Behav. Ecol.* **9**: 592–597.
- Gay, L., Hosken, D.J., Vasudev, R., Tregenza, T. & Eady, P.E. 2009. Sperm competition and maternal effects differentially influence testis and sperm size in *Callosobruchus maculatus*. *J. Evol. Biol.* **22**: 1143–1150.
- Gwynne, D.T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. *Science* **213**: 779–780.
- Gwynne, D.T. 1986. Courtship feeding in katydids (Orthoptera: Tettigoniidae): investment in offspring or in obtaining fertilizations? *Am. Nat.* **128**: 342–352.
- Gwynne, D.T. 1991. Sexual competition among females: what causes courtship-role reversal?. *Trends Ecol. Evol.* **6**: 118–121.
- Gwynne, D.T. 1993. Food quality controls sexual selection in Mormon crickets by altering male mating investment. *Ecology* **74**: 1406–1413.
- Gwynne, D.T. & Brown, W.D. 1994. Mate feeding, offspring investment, and sexual differences in katydids (Orthoptera, Tettigoniidae). *Behav. Ecol.* **5**: 267–272.
- Gwynne, D.T. & Simmons, L.W. 1990. Experimental reversal of courtship roles in an insect. *Nature* **346**: 172–174.
- Hosken, D.J. & Ward, P.I. 2001. Experimental evidence for testis size evolution via sperm competition. *Ecol. Lett.* **4**: 10–13.
- Huignard, J. 1983. Transfer and fate of male secretions deposited in the spermatophore of females of *Acanthoscelides obtectus* Say (Coleoptera Bruchidae). *J. Insect Physiol.* **29**: 55–63.
- Ingleby, F.C., Lewis, Z. & Wedell, N. 2010. Level of sperm competition promotes evolution of male ejaculate allocation in a moth. *Anim. Behav.* **80**: 37–43.
- Jiggins, F.M., Hurst, G.D.D. & Majerus, M.E.N. 2000. Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. *Proc. R. Soc. B* **267**: 69–73.
- Katvala, M., Rönn, J.L. & Arnqvist, G. 2008. Correlated evolution between male ejaculate allocation and female remating behaviour in seed beetles (Bruchidae). *J. Evol. Biol.* **21**: 471–479.
- Kelly, C.D. & Jennions, M.D. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol. Rev.* **86**: 863–884.
- Leimar, O., Karlsson, B. & Wiklund, C. 1994. Unpredictable food and sexual size dimorphism in insects. *Proc. R. Soc. B* **258**: 121–125.
- Lüpold, S., Tomkins, J.L., Simmons, L.W. & Fitzpatrick, J.L. 2014. Female monopolization mediates the relationship between pre- and post-copulatory sexual traits. *Nat. Commun.* **5**: 3184.
- Markow, T.A., Gallagher, P.D. & Krebs, R.A. 1990. Ejaculate-derived nutritional contribution and female reproductive success in *Drosophila mojavensis* (Patterson and Crow). *Funct. Ecol.* **4**: 67–73.
- McNamara, K.B., Elgar, M.A. & Jones, T.M. 2009. Large spermatophores reduce female receptivity and increase male paternity success in the almond moth, *Cadra cautella*. *Anim. Behav.* **77**: 931–936.
- Moya-Laraño, J. & Fox, C.W. 2006. Ejaculate size, second male size, and moderate polyandry increase female fecundity in a seed beetle. *Behav. Ecol.* **17**: 940–946.
- Parker, G.A. & Ball, M.A. 2005. Sperm competition, mating rate and the evolution of ejaculate sizes: a population model. *Biol. Lett.* **1**: 235–238.
- Parker, G.A. & Pizzari, T. 2010. Sperm competition and ejaculate economics. *Biol. Rev.* **85**: 897–934.
- Parker, G.A. & Simmons, L.W. 1989. Nuptial feeding in insects: theoretical models of male and female interests. *Ethology* **82**: 3–26.
- Parker, G.A. & Simmons, L.W. 1996. Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proc. R. Soc. B* **263**: 315–321.
- Parker, G.A., Ball, M.A., Stockley, P. & Gage, M.J.G. 1997. Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. B* **264**: 1793–1802.
- Parker, G.A., Lessells, C.M. & Simmons, L.W. 2013. Sperm competition games: a general model for pre-copulatory male-male competition. *Evolution* **67**: 95–109.
- Perry, J.C. & Rowe, L. 2008. Ingested spermatophores accelerate reproduction and increase mating resistance but are not a source of sexual conflict. *Anim. Behav.* **76**: 993–1000.
- Perry, J.C., Sirot, L. & Wigby, S. 2013. The seminal symphony: how to compose an ejaculate. *Trends Ecol. Evol.* **28**: 414–422.
- Pitnick, S., Markow, T.A. & Riedy, M.F. 1991. Transfer of ejaculate and incorporation of male-derived substances by females in the nannoptera species group (Diptera: Drosophilidae). *Evolution* **45**: 774–780.
- Pitnick, S., Miller, G.T., Reagan, J. & Holland, B. 2001. Males' evolutionary responses to experimental removal of sexual selection. *Proc. R. Soc. B* **268**: 1071–1080.
- Poiani, A. 2006. Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* **60**: 289–310.
- Quinn, J.S. & Sakaluk, S.K. 1986. Prezygotic male reproductive effort in insects: why do males provide more than sperm? *Fla. Entomol.* **69**: 84–94.
- Reuter, M., Linklater, J.R., Lehmann, L., Fowler, K., Chapman, T. & Hurst, G.D.D. 2008. Adaptation to alterations of the operational sex ratio in populations of *Drosophila melanogaster*. *Evolution* **62**: 401–412.
- Robson, L.J. & Gwynne, D.T. 2010. Measuring sexual selection on females in sex-role-reversed Mormon crickets (*Anabrus simplex*, Orthoptera: Tettigoniidae). *J. Evol. Biol.* **23**: 1528–1537.
- Rönn, J.L., Katvala, M. & Arnqvist, G. 2008. Interspecific variation in ejaculate allocation and associated effects on female fitness in seed beetles. *J. Evol. Biol.* **21**: 461–470.
- Sakaluk, S.K. 1984. Male crickets feed females to ensure complete sperm transfer. *Science* **223**: 609–610.
- Sakaluk, S.K. 1986. Is courtship feeding by male insects parental investment? *Ethology* **73**: 161–166.

- Salehialavi, Y., Fritzsche, K. & Arnqvist, G. 2011. The cost of mating and mutual mate choice in 2 role-reversed honey locust beetles. *Behav. Ecol.* **22**: 1104–1113.
- Savalli, U.M. & Fox, C.W. 1998. Sexual selection and the fitness consequences of male body size in the seed beetle *Stator limbatus*. *Anim. Behav.* **55**: 473–483.
- Simmons, L.W. 1992. Quantification of role reversal in relative parental investment in a bush cricket. *Nature* **358**: 61–63.
- Simmons, L.W. & Bailey, W.J. 1990. Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: Tettigoniidae). *Evolution* **44**: 1853–1868.
- Simmons, L.W. & Garcia-Gonzalez, F. 2008. Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles. *Evolution* **62**: 2580–2591.
- Simmons, L.W. & Kvarnemo, C. 1997. Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. *Proc. R. Soc. B* **264**: 1203–1208.
- Simmons, L.W. & Parker, G.A. 1989. Nuptial feeding in insects: mating effort versus paternal investment. *Ethology* **81**: 332–343.
- Smith, R.L. 1979. Paternity assurance and altered roles in the mating behaviour of a giant water bug, *Abedus herberti* (Heteroptera: Belostomatidae). *Anim. Behav.* **27**: 716–725.
- Takakura, K. 1999. Active female courtship behaviour and male nutritional contribution to female fecundity in *Bruchidius dorsalis* (Fahraeus) (Coleoptera: Bruchidae). *Res. Popul. Ecol.* **41**: 269–273.
- Takakura, K. 2001. Courtship-role-reversal in the bean weevil, *Bruchidius dorsalis* (Coleoptera: Bruchidae): interplay between male-male competition and cryptic female choice. *Appl. Entomol. Zool.* **36**: 311–316.
- Takakura, K. 2004. The nutritional contribution of males affects the feeding behavior and spatial distribution of females in a bruchid beetle, *Bruchidius dorsalis*. *J. Ethol.* **22**: 37–42.
- Takakura, K. 2006. Estimation of relative reproductive expenditure in the courtship-role-reversed bean weevil, *Bruchidius dorsalis* (Fahraeus). *J. Ethol.* **24**: 33–36.
- Tallamy, D.W. 1994. Nourishment and the evolution of paternal investment in subsocial arthropods. In: *Nourishment and Evolution in Insect Societies* (J.H. Hunt & C.A. Nalepa, eds), pp. 21–56. Westview Press, Boulder, CO.
- Thornhill, R. 1976. Sexual selection and nuptial feeding behavior in *Bittacus apicalis* (Insecta: Mecoptera). *Am. Nat.* **110**: 529–548.
- Vahed, K. 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biol. Rev.* **73**: 43–78.
- Vahed, K. 2006. Larger ejaculate volumes are associated with a lower degree of polyandry across bushcricket taxa. *Proc. R. Soc. B* **273**: 2387–2394.
- Vahed, K. & Parker, D.J. 2011. The evolution of large testes: sperm competition or male mating rate? *Ethology* **118**: 107–117.
- Wearing-Wilde, J. 1996. Mate choice and competition in the barklouse *Lepinotus patruelis* (Psocoptera: Trogiidae): the effect of diet quality and sex ratio. *J. Insect Behav.* **9**: 599–612.
- Wedell, N. 1991. Sperm competition selects for nuptial feeding in a bushcricket. *Evolution* **45**: 1975–1978.
- Wedell, N. 1996. Mate quality affects reproductive effort in a paternally investing species. *Am. Nat.* **148**: 1075–1088.
- Wedell, N. & Cook, P.A. 1999. Strategic sperm allocation in the Small White butterfly *Pieris rapae* (Lepidoptera: Pieridae). *Funct. Ecol.* **13**: 85–93.
- Wedell, N., Gage, M.J.G. & Parker, G.A. 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* **17**: 313–320.
- Wickler, W. 1985. Stepfathers in insects and their pseudo-parental investment. *Z. Tierpsychol.* **69**: 72–78.
- Wickler, W. 1986. Mating costs versus parental investment: a reply to Gwynne. *Ethology* **71**: 78–79.
- Wigby, S. & Chapman, T. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* **58**: 1028–1037.
- Williams, P.D., Day, T. & Cameron, E. 2005. The evolution of sperm-allocation strategies and the degree of sperm competition. *Evolution* **59**: 492–499.
- Zeh, D.W. & Smith, R.L. 1985. Paternal investment by terrestrial arthropods. *Am. Zool.* **25**: 785–805.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Parameter estimates and test statistics for terms excluded from the model for ejaculate weight (as a percentage of male weight).

Table S2 Parameter estimates and test statistics for terms excluded from the model for lifetime offspring production of females following a single mating.

Table S3 Parameter estimates and test statistics for terms excluded from the model for female lifespan (days) following a single mating.

Table S4 Line means for male and female body size (mg).

Table S5 Male line means for ejaculate weight, relative ejaculate weight and total offspring.

Table S6 Female line means for lifespan and number of offspring produced.

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