

# Do female black field crickets *Teleogryllus commodus* benefit from polyandry?

M. D. JENNIONS,<sup>1</sup> J. M. DRAYTON,<sup>1</sup> R. BROOKS<sup>2</sup> & J. HUNT<sup>3</sup>

<sup>1</sup>School of Botany and Zoology, The Australian National University, Canberra, ACT, Australia

<sup>2</sup>School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia

<sup>3</sup>Centre for Ecology & Conservation, University of Exeter in Cornwall, Penryn, UK

## Keywords:

crickets;  
genetic incompatibility;  
multiple mating;  
polyandry;  
sexual selection;  
sire effects;  
sperm competition;  
*Teleogryllus commodus*.

## Abstract

Female insects that mate multiply tend to have increased lifetime fitness, apparently because of greater access to male-derived resources (e.g. sperm, nuptial gifts) that elevate fertility/fecundity. Experiments that standardize the number of matings per female also show that polyandry can improve aspects of offspring performance, most notably early embryo survival (egg hatching success). This improvement is widely attributed to genetic benefits which would arise if polyandrous females skew paternity to produce fitter offspring. In two separate experiments with field crickets (*Teleogryllus commodus*) polyandrous females (two, three or four mates) did not have higher egg hatching success than monandrous females (effect sizes:  $r = 0.03$  and  $0.08$  for the respective experiments), which is consistent with our finding of no sire effect on hatching success. Polyandry also had no effect on post-hatching offspring survival. Polyandrous females' offspring took significantly longer to mature but their sons were not heavier and their daughters were actually significantly smaller than those of monandrous females. Finally, after controlling for relative male size, monandrous females' sons were more successful when directly competing for a mate.

## Introduction

Why, despite the potential costs, do females in so many species mate with numerous males before breeding (hereafter 'polyandry') (Jennions & Petrie, 2000; Tregenza & Wedell, 2000; Zeh & Zeh, 2003)? In some species, males coerce females into mating more often than is optimal (Arnqvist & Rowe, 2005). In others, however, females actively solicit copulations from numerous males and even discriminate against previous mates (Ivy *et al.*, 2005), suggesting that polyandry is beneficial to them. Indeed, several field studies report a positive relationship between female mating rate and offspring survival (e.g. Madsen *et al.*, 1992), although confounding factors could have an independent causal effect on both variables (e.g. female size, Harano *et al.*, 2006).

Polyandry could be a simple by-product of selection for repeated mating, with no inherent benefit to mating with several males. Repeated mating with the same male often increases female fitness through the acquisition of male-derived materials, such as nutrients or defensive chemicals, that elevate female fecundity and/or longevity (Arnqvist & Nilsson, 2000). Monandry can also reduce fertility if sperm transfer is unsuccessful or incomplete (García-González, 2004) or males are sperm depleted (Preston *et al.*, 2001). This by-product explanation for polyandry is challenged, however, by experiments that follow the protocol of Tregenza & Wedell (1998) in which mating frequency is held constant while the number of mates is varied. In these experiments, polyandry has led to increases in several fitness components (e.g. fecundity: Newcomer *et al.*, 1999; Eady *et al.*, 2001; Worden & Parker, 2001; egg hatching success: Engqvist, 2006; offspring size: Ojanguren *et al.*, 2005; offspring survival: Ivy & Sakaluk, 2005; sons' reproductive success: Bernasconi & Keller, 2001). Although genetic benefits have been invoked to explain this

Correspondence: M.D. Jennions, School of Botany and Zoology, The Australian National University, Canberra ACT 0200, Australia.  
Tel.: +61 2 6125 3540; fax: +61 2 6125 5573;  
e-mail: michael.jennions@anu.edu.au

improved offspring performance it is difficult to eliminate fully any role for synergistic interactions between ejaculates from different males (Simmons, 2001; Engqvist, 2006), or between offspring sired by different males (indirect genetic effects; see Zeh & Zeh, 2006), or maternal effects (Simmons, 2005). Before pursuing these less readily tested explanations, however, it seems prudent first to test whether polyandry generally elevates offspring fitness.

The most widely reported benefit of polyandry is greater early offspring survival. In insects, polyandry significantly increases prehatching embryo survival (meta-analysis: Simmons, 2005). Whether it elevates other fitness components has not been systematically examined, although all published experiments measure several aspects of offspring performance. It should be noted, however, that there is a trend in most sciences for nonsignificant results to remain unpublished (Møller & Jennions, 2001; but see Leimu & Koricheva, 2005) or to take longer to be published (Jennions & Møller, 2002; Trikalinos & Ioannidis, 2005). Even so, very few experiments using the Tregenza & Wedell (1998) design show that polyandry significantly decreases fitness components (in studies by Fedorka & Mousseau, 2002, and Dunn *et al.*, 2005, polyandrous females died sooner). Interestingly, however, there is evidence from studies with other designs that polyandry reduces female lifetime fecundity (e.g. Orsetti & Rutowski, 2003; Brown *et al.*, 2004; Bybee *et al.*, 2005; Byrne & Rice, 2005). To test whether observed levels of polyandry are an adaptive female tactic or a response to sexual conflict over mating rates, we need to measure the effect of mating rate on a wide range of fitness components and, ultimately, integrate these measures to obtain an estimate of net fitness (Hunt *et al.*, 2004; e.g. Dunn *et al.*, 2005; Head *et al.*, 2005).

### Genetic benefits of polyandry

Polyandry offers genetic benefits if post-mating mechanisms bias paternity towards males that increase offspring fitness (Yasui, 1998). For heuristic reasons, these benefits are attributed to paternity being biased towards males that elevate offspring performance for the majority of females ('intrinsic male quality' hypothesis) or against males where paternal-maternal genome interactions lower offspring performance ('genetic compatibility' hypothesis). In the former, paternity should consistently be biased to males with high breeding values for fitness; in the latter, the favoured male will depend on the female's genotype. These hypotheses correspond with the contrast between additive (e.g. 'good gene') and nonadditive (e.g. compatibility due to epistasis or dominance) genetic effects (Lynch & Walsh, 1998). Genetic variation in traits is almost never exclusively because of either additive or nonadditive effects and well-designed studies are needed to estimate their relative importance.

To date, quantitative genetic analyses of species where females actively mate multiply are rare. Only one study has formally tested for significant additive genetic variation among sires in their effect on prehatching embryo survival (García-González & Simmons, 2005).

In many polyandry studies, several males are each mated to two females and a nonsignificant intraclass correlation (i.e. low repeatability) for egg hatching success within males is taken to mean that there is no intrinsic sire effect for this trait (e.g. Tregenza & Wedell, 1998). The default is then to conclude that any benefits of polyandry attributable to genetic effects are because of paternity being biased towards males that are genetically more compatible (reviewed by Simmons, 2001). This conclusion should be treated carefully, however, as statistical power to detect additive genetic variation depends on the number of females per male (Lynch & Walsh, 1998). It is also noteworthy that this type of sire repeatability experiment is actually a full-sib/half-sib breeding design for a threshold trait (whether or not an egg hatches). Although rarely done, these data can therefore be used to estimate the heritability of a continuous underlying liability trait that influences hatching success with the Crittenden-Falconer method (Roff, 1997, p. 52–61; Lynch & Walsh, 1998, equation 25.1).

Here, in two separate experiments, we test whether polyandry increases various components of offspring fitness in the Australian black field cricket *Teleogryllus commodus*. In two further experiments, we then calculate the heritability of egg hatching success. Female *T. commodus* readily mate multiply under semi-natural and laboratory conditions (Evans, 1988; Jennions *et al.*, 2004). Males do not produce a nutrient-rich spermatophylax, and the spermatophore is < 1% of body mass (Evans, 1988). There is no evidence that, aside from ensuring an adequate supply of sperm, females gain direct benefits from mating repeatedly. The only other obvious benefit of mating multiply in *T. commodus* is to create an opportunity to bias paternity towards males that sire fitter offspring. This benefit is worth testing for as polyandry increased embryo survival in the congeneric *T. oceanicus* (Simmons, 2001).

### Materials and methods

We collected > 120 field-mated females in Canberra, Australia, in February–March 2002 to establish a breeding stock. The stock was maintained in six to eight large tanks per generation at 26–28 °C with a 12 : 12 photoperiod. Summary statistics are presented as mean ± SE.

#### Polyandry: one vs. two vs. four males

Our experimental design followed Tregenza & Wedell (1998) and consisted of 20 experimental blocks of seven females and four males. Each female mated four times: either to the same male ( $n = 4$  females per block), twice

each to two different males ( $n = 2$  females per block) or once each to all four males ( $n = 1$  female per block). Each male mated with one female per mating treatment and mating order was randomized across treatments. After mating we kept the pair together for 60 min to prevent spermatophore removal by the female and ensure complete sperm transfer (Bussière *et al.*, 2006). Each female mated at most once per day. Two females assigned four males refused to mate with all of them. These females were provided with one or two stock males and they eventually mated with four different males. Seven females in the monandry treatment refused to mate four times (five mated twice and two thrice). Their egg hatching success did not differ from that of monogamous females mating four times ( $t_{78} = 1.32$ ,  $P = 0.23$ ). We included these females in the analyses as their exclusion did not alter our findings and previous work shows that there is no effect on egg hatching success of varying the number of matings with the same male (M.D. Jennions, unpublished data). Mating trials started on day 1 with 80 experimental males (age: > 10 days) randomly assigned to blocks and 140 virgin females (age: 10–21 days) randomly assigned to mating treatments. Males were mated to a stock female the previous day to ensure that they were sexually competent.

All mating trials were completed by day 7. Females were then placed in individual containers ( $17 \times 12 \times 7$  cm) with some egg carton, cat food, water and a dish of moist sand for egg-laying. After a week, the sand dish was removed and replaced. Each week we counted out up to 100 eggs and transferred them onto moist cotton wool in a Petri dish in a sealed container. We used these two sets of eggs per female to test whether hatching success was significantly repeatable among females across weeks. We checked containers for hatchlings every 2–3 days for 50 days and opportunistically thereafter for a month. Of the 140 females, 131 produced more than 100 eggs and 139 produced sufficient eggs (> 20) to estimate hatching success.

We then reared hatchlings to adulthood to obtain information on life-history traits (e.g. development time and adult size). On day 35, we transferred up to 100 hatchlings per female into 2.5-L containers with *ad libitum* food and water. We set up 100 hatchlings per female for 75 females and fewer for those that did not produce as many hatchlings (> 50 hatchlings per female for 34 females and 10–49 hatchlings per female for 22 females). Containers were cleaned weekly and offspring number counted. The sexes were separated as soon as they could be identified. We recorded the week in which each adult matured and adult body mass ( $\pm 0.1$  mg). We used two measures of offspring survival: (a) from hatching to maturity ( $45.5 \pm 1.3\%$ ); and (b) from 4 weeks after being set up in containers to maturation ( $70.3 \pm 1.1\%$ ). The latter measure was included in case new hatchlings were more susceptible to stochastic events that result in high mortality.

Finally, we compared the mating ability of sons when they competed for a mate. We placed a stock female and the son of a monogamous and of a polyandrous (four mates) female from the same experimental block in a  $17 \times 12 \times 7$  cm<sup>3</sup> container. We then noted which male was the first to successfully transfer a spermatophore ( $n = 120$  contests with males from 17 blocks).

### Polyandry: one vs. three mates

We performed another polyandry experiment in 2003. Females mated three times, either to the same male ( $n = 60$ ) or to three different males ( $n = 76$ ). Each male mated with only one female. We measured egg hatching success based on 100 eggs for 116 females and > 53 eggs for 20 females. Some eggs hatched for 98.5% of the females. The experiment was conducted with six sets of females over several months so we included 'time block' as a random factor in our analyses to control for any temporal sources of variation in hatching success.

### Sire effect on hatching success

We estimated the heritability of hatching success in two separate studies. In February 2004, 39 field-caught males were each assigned three virgin stock females and allowed to mate twice with each (four females refused a second mating). For three females that initially laid no eggs, a third mating was provided. We measured body size (hind leg length) and recorded whether a female was a male's first, second or third mate. Females were then housed individually. After a week, if possible, each female's clutch was divided into two similar-sized sets of eggs ( $82.5 \pm 1.4$ ;  $n = 202$  sets). Of the 107 females, three died prior to egg laying, six yielded one set of eggs and 104 females produced enough eggs (> 20) to estimate hatching rate. Repeatability of hatching success among females was calculated for the 98 females with two sets of eggs. We monitored emergence of hatchlings every second day for 166 days.

In June 2004, 12 unrelated males (the sons of different wild-caught females) were each assigned 10 or 11 virgin stock females. We obtained one set of eggs per female ( $89.5 \pm 1.6$  eggs). Of 102 females that laid sufficient eggs (> 20) to estimate hatching rate, 93.1% had some hatch ( $7.9 \pm 0.6$  usable females per male). To maximize the number of females per male, females were initially mated once. Two females were re-mated because they did not lay eggs after the first mating, indicating probable failed sperm transfer. After re-mating both oviposited. Hatchling emergence was recorded every day for 120 days.

### Statistical analysis

#### *Polyandry experiments*

Egg hatching success is a proportion, so we ran generalized linear mixed models (GLMM) with binomial error

using the *glmmPQL* function in S-Plus 7.0 with model parameters estimated using a maximum likelihood approach (Venables & Ripley, 2002). In the first experiment, 'block' was a random factor and 'mating treatment' a fixed factor. Egg hatching success was repeatable among females between weeks ( $r_I = 0.56$ ,  $F_{120,121} = 3.40$ ,  $P < 0.001$ ,  $n = 121$ ), so we pooled the weekly data. Separate analyses for each week produced the same results. For pairwise comparisons, GLMM present  $P$ -values from  $t$ -tests using the standard error of the difference between a focal group of the categorical fixed factor and other groups (Crawley, 2002). We set either the monandry or polyandry (four males) treatment as the focal group (see Table 1). In the second experiment, 'mating treatment' was a fixed factor and 'time block' was a random factor.

To analyse variation in offspring performance, we ran linear mixed models in S-Plus with 'block' as a random factor. 'Female identity' was included as a random factor for the analysis of offspring mass because multiple offspring per female were measured. For other variables there was a single measure per female (e.g. sex ratio). Model simplification proceeded by backward elimination. Initial models included the fixed term 'female mating type' and an estimate of 'rearing density', which was the number of offspring alive after 4 weeks. 'Rearing density' correlated with the initial number of hatchlings set up ( $r = 0.64$ ,  $n = 131$ ,  $P < 0.0001$ ), but seemed a better estimate of effective density because of occasional high initial mortality of first instar hatchlings. Nonsignificant terms were excluded from final models but the  $P$ -value for each, if included in the model, is provided. The response variables examined were adult body mass and mean time to maturity of each sex, offspring sex ratio and the two measures of offspring survival. We used mean time to maturity rather than individual values in the analysis because we counted the number of offspring maturing each week. Thus, although the mean is a reasonable summary value for each female, values for individual offspring are imprecise.

Finally, to test for an effect of mating treatment on the mating success of sons, we ran a GLMM with binomial error with fight outcome (whether or not the polyandrous female's son mated) as the dependent variable, 'block' as a random factor and the size difference (polyandry – monandry) between the two competing males as a continuous fixed factor. We tested whether the intercept differed significantly from zero. This tests whether there is an equal probability of the polyandrous or monandrous females' son winning a contest when the males are size-matched (i.e. size difference is zero) because a log-odds ratio of zero is equivalent to equal odds.

#### Sire effect on hatching success

Hatching success was highly repeatable across two sets of eggs from the same male–female pairing ( $r_I = 0.82$ ,

**Table 1** Summary statistics and parameter estimates (mean  $\pm$  SE) from linear mixed models with 'mating treatment' as a fixed factor and 'block' as a random factor (for offspring mass, 'female identity' was also included as a random factor).

Variable	Mating treatment	Intercept	Monogamy	Polyandry (2 males)	Polyandry (4 males)	Rearing density (offspring alive at week 4)
Hatching success*	$F_{2,117} = 0.222$ , $P = 0.80$	$-0.829 \pm 0.067$	0	$0.054 \pm 0.097$	$-0.027 \pm 0.129$	not applicable
Survival (hatching to adult)*	$F_{2,108} = 0.438$ , $P = 0.65$	$1.483 \pm 0.176$	$-0.071 \pm 0.047$	$0.006 \pm 0.041$	0	$-0.011 \pm 0.003$ ( $F_{1,108} = 37.51$ , $P < 0.001$ )
Survival (week 4 to adult)*	$F_{2,108} = 1.041$ , $P = 0.36$	$-1.111 \pm 0.156$	$-0.020 \pm 0.051$	$0.047 \pm 0.044$	0	$0.015 \pm 0.002$ ( $F_{1,108} = 18.05$ , $P < 0.001$ )
Sons' development time (days)†	$F_{2,106} = 7.104$ , $P < 0.001$	$90.2 \pm 1.9$	0	$+4.2 \pm 1.4$	$+5.4 \pm 1.8$	$-0.092 \pm 0.031$ ( $F_{1,106} = 8.922$ , $P = 0.004$ )
Daughters' development time (days)†	$F_{2,107} = 3.121$ , $P = 0.048$	$84.3 \pm 0.8$	0	$+2.7 \pm 1.3$	$+3.1 \pm 1.7$	( $F_{1,106} = 0.582$ , $P = 0.45$ )
Son's mass (mg)	$F_{2,108} = 0.39$ , $P = 0.68$	$626.2 \pm 22.3$	0	$-7.9 \pm 13.9$	$-13.6 \pm 18.8$	( $F_{1,430} = 1.202$ , $P = 0.27$ )
Daughter's mass (mg)	$F_{2,106} = 4.26$ , $P = 0.017$	$937.5 \pm 21.6$	0	$-38.7 \pm 15.1$	$-31.6 \pm 19.9$	$-1.54 \pm 0.35$ ( $F_{1,106} = 19.41$ , $P < 0.001$ )
Sex ratio (female : male)	$F_{2,106} = 0.493$ , $P = 0.61$	$1.47 \pm 0.080$	0	$-0.13 \pm 0.133$	$-0.093 \pm 0.18$	( $F_{1,105} = 3.14$ , $P = 0.08$ )

Rearing density was only included in the final model if it has a significant effect (see text for details).

\*Logit-transformation of the proportion  $p$  hatching or surviving,  $p = e^{(a+x)} / (1 + e^{(a+x)})$ , where  $a$  = intercept,  $x$  = parameter value for mating treatment.

†The number of days from when the female was first provided with sand until the mean date of hatching emergence.

$F_{97,98} = 10.80$ ,  $P < 0.001$ ,  $n = 98$ ) so we pooled the sets. We estimated the heritability of hatching success as a threshold trait with an underlying normally distributed liability following the Crittenden–Falconer method (Lynch & Walsh, 1998, eq. 25.1a). We used *PopTools* (*v*) to resample hatching success across families with replacement to establish a distribution of 1000 pseudo-estimates of  $h_s^2$ . We then calculated the proportion of pseudo-estimates that were equal to or larger than the observed value. We also calculated 95% confidence intervals for  $h_s^2$  by obtaining 1000 bootstrap estimates of  $h_s^2$  in which we sampled (with replacement) at random 80% of full-sib families and calculated  $h_s^2$  on this reduced sample. To test whether ‘female size’ or ‘mating order’ explained variation in the proportion of eggs that hatched or mean hatching time, we ran a linear mixed model with ‘male identity’ as the random factor.

In the first study, some eggs hatched for 96% of sets (194 of 202). Only two of 99 females had no eggs hatch in both sets of eggs, thus for only four of 202 sets could complete hatching failure possibly be attributed to failed sperm transfer. In the second study, some eggs hatched for 95 of 102 females. It is possible that complete hatching failure is because of a lack of sperm rather than genetic effects. We therefore took a conservative approach and excluded females for whom no eggs hatched. Their inclusion produced nearly identical results. Unless otherwise stated, tests are two-tailed,  $\alpha = 0.05$  and summary statistics are mean  $\pm$  SE.

## Results

### Polyandry: one vs. two vs. four males

Summary and test statistics are provided in Table 1. For ease of interpretation, back-calculated treatment mean values are presented in the text where appropriate (e.g. of logit transformed proportions). There was no significant difference in hatching success among mating treatments (1, 2 or 4 mates: 30.4%, 31.5%, 29.8%). Likewise, the proportion of offspring that survived from week 4 to adulthood or from hatching to adulthood did not differ among mating treatments. Both declined significantly as rearing density increased. There was no effect of mating treatment or offspring rearing density on the adult sex ratio.

The mean time to maturity for daughters differed significantly among mating treatments, but it did not depend on rearing density. Monogamous females’ daughters matured sooner than those of females mated to two males ( $P = 0.04$ ) or to four males ( $P = 0.06$ ). The mean time to maturity for sons also differed significantly among treatments. Monogamous females’ sons matured sooner than those of females mated to two males ( $P = 0.003$ ) or to four males ( $P = 0.003$ ). In addition, males matured significantly sooner when rearing density was higher.

There was no significant difference among mating treatments in the adult mass of sons (1, 2 or 4 mates: 649, 640, 634 mg), nor any effect of rearing density. There was, however, a significant difference among mating treatments in the mean mass of daughters and a significant decline in mass with greater offspring rearing density. On average, the daughters of monogamous females were significantly heavier than those of females mated to two males ( $P = 0.012$ ) but not significantly heavier than those of females mated to four males ( $P = 0.12$ ) (1, 2 or 4 mates: 860, 822, 829 mg at a rearing density of 50 offspring).

When the son of a polyandrous female and a monandrous female competed for mating the size difference between them had a nonsignificant effect on who won the contest ( $t_{99} = 1.67$ ,  $P = 0.098$ ). The larger male transferred a spermatophore first in 68 of 118 contests. The intercept value was significantly less than zero ( $-0.443 \pm 0.198$ ;  $t_{99} = 2.23$ ,  $P = 0.028$ ) which indicates that when the males were size-matched the polyandrous female’s son had only a 39.1% chance of winning the contest. Thus, monandrous females’ sons were more successful when competing for mates.

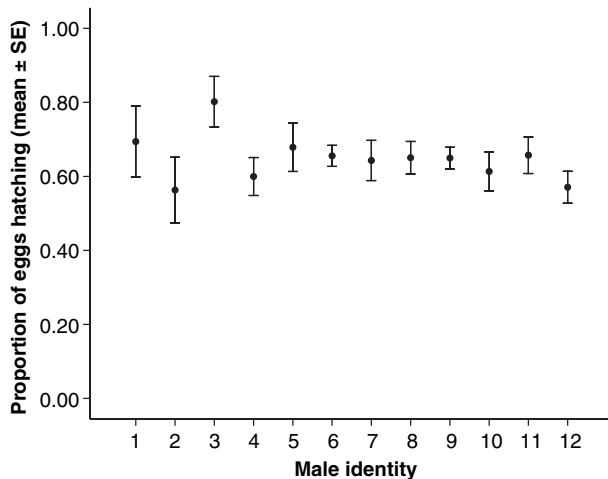
### Polyandry: one vs. three males

There was no significant difference in egg hatching success between monandrous and polyandrous females ( $F_{1,129} = 1.322$ ,  $P = 0.250$ ; logit transformation of proportion hatching:  $a + bx = -0.214 \pm 0.240 + 0$  and  $-0.214 \pm 0.240 + 0.156 \pm 0.135$ ; back-transformed mean values = 44.7% vs. 48.5% for monandrous and polyandrous females respectively).

### Sire effects on hatching success

For 39 wild-caught males, there was no effect of female size ( $F_{1,59} = 1.94$ ,  $P = 0.17$ ) or mating order ( $F_{1,59} = 0.239$ ,  $P = 0.63$ ) on hatching success. Egg hatching success for the 99 females that produced some hatchlings was  $72.7 \pm 1.7\%$ . The heritability of hatching success for an underlying continuous liability trait that influences whether or not an egg reaches a threshold and hatches was effectively zero. There was clearly no sire effect on egg hatching success.

The statistically more powerful second study also showed no sire effect on hatching success ( $P = 0.922$ ) and  $h_s^2$  was  $-0.002$  (95% CI:  $-0.13$ ,  $0.10$ ) (Fig. 1). There was, however, a small but significant decline in hatching success with mating order ( $F_{1,82} = 5.415$ ,  $P = 0.02$ ,  $n = 95$ ) that might indicate partial sperm depletion or a male age effect. This decline was not apparent over the first eight matings ( $P = 0.16$ ,  $n = 82$ ). Hatching success for the 95 females for whom some eggs hatched was  $63.7 \pm 1.6\%$ . Finally, there was a significant sire effect on mean hatching time ( $h_s^2 = 0.47 \pm 0.40$ ,  $P = 0.033$ ).



**Fig. 1** The proportion of eggs hatching (mean  $\pm$  SE) for 12 males that mated with several females each ( $n = 4, 9, 4, 10, 6, 9, 9, 8, 8, 9, 11, 8$  females).

## Discussion

In insects multiple mating generally increases lifetime fecundity even though it can reduce female longevity (Arnqvist & Nilsson, 2000) and this direct benefit is widely attributed to increased access to male-derived material resources transferred during mating. Experiments that control the number of matings but vary the number of mates also show that polyandry can improve offspring performance (e.g. Tregenza & Wedell, 1998). This improvement suggests that there are genetic benefits to polyandry because post-mating mechanisms bias paternity towards males that sire fitter offspring (Jennions & Petrie, 2000). It is, however, also possible that maternal effects (e.g. Tregenza *et al.*, 2003; Kozielska *et al.*, 2004) or indirect genetic effects (e.g. Zeh & Zeh, 2006) play a role (review: Simmons, 2005).

### Polyandry and egg hatching success

The most widely reported genetic benefit of polyandry in arthropods is increased early embryo survival (i.e. egg hatching success) (Simmons, 2005). To date, six experiments have reported that polyandry significantly elevates early offspring survival (Tregenza & Wedell, 1998; Newcomer *et al.*, 1999; Simmons, 2001; Fedorka & Mousseau, 2002; Engqvist, 2006; Fisher *et al.*, 2006b), whereas seven have not (Watson, 1998; Baker *et al.*, 2001; Worden & Parker, 2001; Kamimura, 2003; Tregenza *et al.*, 2003; Dunn *et al.*, 2005; Ivy & Sakaluk, 2005). Excluding our study, the average effect of polyandry on egg hatching success is  $r \approx 0.20$  (from Simmons, 2005;  $n = 79 \pm 10$  females per study). Polyandry did not elevate hatching success in *T. commodus* in either of our experiments. We estimate the mean effect

as  $r = 0.03$  (95% CI:  $-0.14$  to  $0.20$ ) and  $0.08$  (95% CI:  $-0.09$  to  $0.25$ ) for the one vs. three and four mate comparisons respectively. Despite sample sizes almost double the average for polyandry experiments that control for mating rate ( $n = 140, 136$  females) our statistical power to detect a significant effect if  $r = 0.2$  is still only  $\sim 66\%$ . However, we obtained similarly low estimates of the effect size from both our experiments, so if there is any genetic benefit to polyandry in *T. commodus* it is probably small.

### Sire effects on hatching success

Hatching success (early embryo survival) is the only trait for which there is general experimental evidence that polyandry improves offspring performance. To what extent, however, do males vary predictably in their effect on hatching success? One approach is to estimate heritability from a full-sib/half-sib breeding design that tests whether sire identity explains a significant amount of variation in sons' effects on hatching success (e.g. García-González & Simmons, 2005). A more familiar approach that has been used in many polyandry studies is to assign males two females each and then test whether egg hatching success is significantly repeatable among males. Although it has not been explicitly stated in previous studies, this is actually a full-sib/half-sib breeding design. Repeatability of a trait for offspring from different females because of sire effects is quantified as the sire intraclass correlation ( $t_s$ ) from a nested ANOVA. Heritability is then  $4t_s$  (Roff, 1997, equation 2.32). If we treat hatching success as a threshold trait it is possible to estimate the heritability of the underlying liability trait influencing success using the Crittenden–Falconer method (Roff, 1997; Lynch & Walsh, 1998). In two separate studies, our estimates of heritability of egg hatching success were both very close to zero.

As with any genetic study, low heritability might be due to large measurement error, high levels of environmentally-induced variation, strong maternal or non-additive genetic effects or genuinely low levels of additive genetic variation. Published studies have failed to find a repeatable male effect on hatching success, but most of these studies had low statistical power (Simmons, 2005). In *T. commodus* there was no evidence that sire identity explained variation in hatching success. This finding cannot readily be attributed to measurement error or environmental influences during rearing as hatching success was highly repeatable for eggs collected from the same pair between weeks ( $r_t = 0.56$ ,  $P < 0.001$ ,  $n = 121$ ) or eggs collected at the same time but incubated in separate containers ( $r_t = 0.82$ ,  $P < 0.001$ ,  $n = 98$ ) (see also Jennions *et al.*, 2004). There is some evidence for strong maternal effects. In another full-sib/half-sib study of *T. commodus* we documented strong maternal effects on post-hatching offspring performance, even though most traits show very low heritability (M.D. Jennions,

unpublished data). Maternal effects could be due to environmental factors or nonadditive genetic effects (e.g. well-fed or heterozygous females invest more into each egg).

Our second study, with up to 10 dams per sire, is statistically more powerful than our first test and all previous studies whose intraclass correlations are calculated based on two or three females per male. For a given total sample size the optimal experimental design is mainly dependent on increasing the number of females per male (Lynch & Walsh, 1998). Even so, our second study only had a 28% chance of detecting a medium strength effect (*sensu* Cohen, 1988). Nonetheless, our two heritability estimates suggest that if there is additive genetic variation for hatching success then it is very small relative to phenotypic variation. Of course, there might be substantial nonadditive genetic effects on hatching success because of strong interactions between paternal and maternal genotypes but calculating any such effects requires highly complex breeding designs. Based on the extreme case of inbreeding avoidance, however, *T. commodus* females do not seem to bias paternity towards genetically more compatible mates (Jennions *et al.*, 2004). Finally, it is worthwhile noting that our experimental design was sufficiently powerful to show that mean hatching time was significantly heritable ( $P = 0.03$ ).

### Polyandry and offspring fitness

Most studies that test whether polyandry is beneficial measure several aspects of offspring performance, such as body size, development time and post-hatching survival. Unfortunately, there is still insufficient data to conduct meta-analyses for most of these traits. A preliminary survey indicates that in seven of 29 tests from experimental studies, polyandrous females' offspring performed significantly better (larger body size: Watson, 1998; Ivy & Sakaluk, 2005; Fisher *et al.*, 2006a; faster development: Watson, 1998; greater offspring survival: Ivy & Sakaluk, 2005; Fisher *et al.*, 2006b; higher reproductive success for sons: Bernasconi & Keller, 2001). Studies that fail to explicitly control for mating rate also suggest that there could be additional benefits to polyandry but these benefits cannot be unambiguously attributed to genetic effects (e.g. anti-predator behaviour: Evans & Magurran, 2000; daughters' fecundity: Konior *et al.*, 2001).

In our study of *T. commodus* polyandry did not affect offspring survival or the sex ratio at maturation. Unexpectedly, monogamous females' offspring matured significantly sooner but this was not at the cost of reduced adult size as their daughters were significantly heavier ( $P = 0.02$ ) and their sons were no different in mass to those of polyandrous females. When males competed directly for a mating, polyandrous female's sons were significantly less likely to win an encounter when males were size matched. In the field cricket *Gryllus bimaculatus* males that won when directly competing for a female

were more likely to produce successful sons, suggesting that this trait is heritable (Wedell & Tregenza, 1999). The greater success of monandrous female's sons that we observed is therefore puzzling and is unlikely to be because of additive genetic effects. Estimating polyandry's effect on net fitness remains a future challenge, especially if fecundity trades off with offspring quality (Hunt *et al.*, 2004). Dunn *et al.* (2005) have, however, recently shown that polyandrous seaweed flies *Coelopa frigida* produce significantly more offspring that survive to adulthood, which suggests that polyandry can indeed increase females' net fitness in some species.

### Concluding remarks

Polyandry did not improve offspring performance in *T. commodus*. Despite some opposition to replicating studies (reviews: Palmer, 2000; Kelly, 2006), it is necessary to confirm the now oft-stated claim, based primarily on evidence for increased hatching success, that polyandry improves *net* offspring performance. The evidence for this benefit is still very limited compared with far stronger evidence that multiple mating increases lifetime fecundity (Arnqvist & Nilsson, 2000), presumably because of direct material rather than indirect genetic benefits.

### Acknowledgments

We thank J. Davies, R. Graham, J. Inggs, J. McInnes, M. Shackleton, Y.N. Su, B. Wong and J. Woodman for assistance. We thank P. Backwell, T. Ivy, D. Fisher and J. Burchell for useful discussions and J. Koricheva for information on publication bias. Our research was funded by Australian Research Council Discovery grants to MDJ, JH and RB.

### References

- Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**: 145–164.
- Arnqvist, G. & Rowe, R. 2005. *Sexual Conflict*. Princeton University Press, Princeton, NJ.
- Baker, R.H., Ashwell, R.I.S., Richards, T.A., Fowler, K., Chapman, T. & Pomiankowski, A. 2001. Effects of multiple mating and male eye span on female reproductive output in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. *Behav. Ecol.* **12**: 732–739.
- Bernasconi, G. & Keller, L. 2001. Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. *J. Evol. Biol.* **14**: 186–193.
- Brown, W.D., Bjork, A., Schneider, K. & Pitnick, S. 2004. No evidence that polyandry benefits females in *Drosophila melanogaster*. *Evolution* **58**: 1242–1250.
- Bussière, L.F., Hunt, J., Jennions, M.D. & Brooks, R. 2006. Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. *Evolution* **60**: 792–800.
- Bybee, L.F., Millar, J.G., Paine, T.D., Campbell, K. & Hanlon, C.C. 2005. Effects of single vs. multiple mates: monogamy

- results in increased fecundity for the beetle *Phoracantha semipunctata*. *J. Insect Behav.* **18**: 513–527.
- Byrne, P.G. & Rice, W.R. 2005. Remating in *Drosophila melanogaster*: an examination of the trading-up and intrinsic male-quality hypotheses. *J. Evol. Biol.* **18**: 1324–1331.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Erlbaum, Hillsdale, NJ.
- Crawley, M.J. 2002. *Statistical Computing: An Introduction to Data Analysis Using S-Plus*. John Wiley & Sons, Chichester.
- Dunn, D.W., Sumner, J.P. & Goulson, D. 2005. The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae). *Behav. Ecol. Sociobiol.* **58**: 128–135.
- Eady, P.E., Wilson, N. & Jackson, M. 2001. Copulating with multiple mates enhances female fecundity but not egg-to-adult survival in the bruchid beetle *Callosobruchus maculatus*. *Evolution* **54**: 2161–2165.
- Engqvist, L. 2006. Females benefit from mating with different males in the scorpionfly *Panorpa cognata*. *Behav. Ecol.* **17**: 435–440.
- Evans, A.R. 1988. Mating systems and reproductive strategies in three Australian Gryllid crickets: *Bobilla victoriae* Otte, *Balamara gidya* Otte and *Teleogryllus commodus* (Walker) (Orthoptera: Gryllidae; Nemobiinae; Trigonidiinae; Gryllinae). *Ethology* **78**: 21–52.
- Evans, J.P. & Magurran, A.E. 2000. Multiple benefits of multiple mating in guppies. *Proc. Natl Acad. Sci. U.S.A.* **97**: 10074–10076.
- Fedoraka, K.M. & Mousseau, T.A. 2002. Material and genetic benefits of female multiple mating and polyandry. *Anim. Behav.* **64**: 361–367.
- Fisher, D.O., Double, M.C. & Moore, B.D. 2006a. Number of mates and timing of mating affect offspring growth in the small marsupial *Antechinus agilis*. *Anim. Behav.* **71**: 289–297.
- Fisher, D.O., Double, M.C., Blomberg, S.P., Jennions, M.D. & Cockburn, A. 2006b. Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature* **444**: 89–92.
- García-González, F. 2004. Infertile matings and sperm competition: The effect of 'nonsperm representation' on intraspecific variation in sperm precedence patterns. *Am. Nat.* **164**: 457–472.
- García-González, F. & Simmons, L.W. 2005. The evolution of polyandry: intrinsic sire effects contribute to embryo viability. *J. Evol. Biol.* **18**: 1097–1103.
- Harano, T., Yasui, Y. & Miyatake, T. 2006. Direct effects of polyandry on female fitness in *Callosobruchus chinensis*. *Anim. Behav.* **71**: 539–548.
- Head, M.L., Hunt, J., Jennions, M.D. & Brooks, R.C. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. *PLOS Biol.* **3**: 289–294.
- Hunt, J., Bussière, L., Jennions, M.D. & Brooks, R.C. 2004. What is genetic quality? *Trends Ecol. Evol.* **19**: 329–333.
- Ivy, T.M. & Sakaluk, S.K. 2005. Polyandry promotes enhanced offspring survival in decorated crickets. *Evolution* **59**: 152–159.
- Ivy, T.M., Weddle, C.B. & Sakaluk, S.K. 2005. Females use self-referent cues to avoid mating with previous mates. *Proc. R. Soc. Lond. B* **272**: 2475–2478.
- Jennions, M.D. & Møller, A.P. 2002. Relationships fade with time: a meta-analysis of temporal trends in publication in ecology and evolution. *Proc. R. Soc. Lond. B* **269**: 43–48.
- Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**: 21–64.
- Jennions, M.D., Hunt, J., Graham, R. & Brooks, R. 2004. No evidence for inbreeding avoidance through postcopulatory mechanisms in the black field cricket, *Teleogryllus commodus*. *Evolution* **58**: 2472–2477.
- Kamimura, Y. 2003. Effects of repeated mating and polyandry on the fecundity, fertility and maternal behaviour of female earwigs, *Euborellia plebeja*. *Anim. Behav.* **65**: 205–214.
- Kelly, C.D. 2006. Replicating empirical research in animal behaviour: how and why it should be done but rarely ever is. *Q. Rev. Biol.* **81**: 221–236.
- Konior, M., Radwan, J. & Kolodziejczyk, M. 2001. Polyandry increases offspring fecundity in the bulb mite. *Evolution* **55**: 1893–1896.
- Kozielska, M., Krzemińska, A. & Radwan, J. 2004. Good genes and the maternal effects of polyandry on offspring reproductive success in the bulb mite. *Proc. R. Soc. Lond. B* **271**: 165–170.
- Leimu, R. & Koricheva, J. 2005. What determines the citation frequency of ecological papers. *Trends Ecol. Evol.* **20**: 28–32.
- Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.
- Møller, A.P. & Jennions, M.D. 2001. Testing and adjusting for publication bias. *Trends Ecol. Evol.* **16**: 580–586.
- Madsen, T., Shine, R., Loman, J. & Håkansson, T. 1992. Why do female adders copulate so frequently? *Nature* **355**, 440–441.
- Newcomer, S.D., Zeh, J.A. & Zeh, D.W. 1999. Genetic benefits enhance the reproductive success of polyandrous females. *Proc. Natl Acad. Sci. U.S.A.* **96**: 10236–10241.
- Ojanguren, A.F., Evans, J.P. & Magurran, A.E. 2005. Multiple mating influences offspring size in guppies. *J. Fish Biol.* **67**: 1184–1188.
- Orsetti, D.M. & Rutowski, R.L. 2003. No material benefits, and a fertilization cost, for multiple mating by female leaf beetles. *Anim. Behav.* **66**: 477–484.
- Palmer, A.R. 2000. Quasireplication and the contract of error: lessons from sex ratios, heritabilities and fluctuating asymmetry. *Annu. Rev. Ecol. Evol. Syst.* **31**: 441–480.
- Preston, B.T., Stevenson, I.R., Pemberton, J.M. & Wilson, K. 2001. Dominant rams lose out by sperm depletion. *Nature* **409**: 681–682.
- Roff, D.A. 1997. *Evolutionary Quantitative Genetics*. Chapman & Hall, New York.
- Simmons, L.W. 2001. The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses. *J. Evol. Biol.* **14**: 585–594.
- Simmons, L.W. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Annu. Rev. Ecol. Evol. Syst.* **36**: 125–146.
- Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. *Evolution* **52**: 1726–1730.
- Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* **9**: 1013–1027.
- Tregenza, T., Wedell, N., Hosken, D.J. & Wards, P.I. 2003. Maternal effects on offspring depend on female mating pattern and offspring environment in yellow dung flies. *Evolution* **57**: 297–304.
- Trikalinos, T.A. & Ioannidis, J.P.A. 2005. Assessing the evolution of effect sizes over time. In: *Publication Bias in Meta-analysis: Prevention, Assessment and Adjustments* (H. R. Rothstein, A. J. Sutton & M. Borenstein, eds), pp. 241–259. John Wiley, New York.
- Venables, W.N. & Ripley, B.D. 2002. *Modern Applied Statistics with S*, 4th edn. Springer Verlag, New York.



- Watson, P.J. 1998. Multi-male mating and female choice increase offspring growth in the spider *Neriene litigiosa* (Linyphiidae). *Anim. Behav.* **55**: 387–403.
- Wedell, N. & Tregenza, T. 1999. Successful fathers sire successful sons. *Evolution* **53**: 620–625.
- Worden, B.D. & Parker, P.G. 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? *Behav. Ecol.* **12**: 761–767.
- Yasui, Y. 1998. The 'genetic benefits' of female multiple mating reconsidered. *Trends Ecol. Evol.* **13**: 246–250.
- Zeh, J.A. & Zeh, D.W. 2003. Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. *Ethology* **109**: 929–950.
- Zeh, J.A. & Zeh, D.W. 2006. Outbred embryos rescue inbred half-siblings in mixed-paternity broods of live-bearing females. *Nature* **439**: 201–203.

*Received 7 December 2006; revised 6 February 2007; accepted 6 February 2007*