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MATERNAL EFFECTS, BUT NO GOOD OR COMPATIBLE GENES FOR SPERM COMPETITIVENESS IN AUSTRALIAN CRICKETS

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Explanations for the evolution of polyandry often center on the idea that females garner genetic benefits for their offspring by mating multiply. Furthermore, postcopulatory processes are thought to be fundamental to enabling polyandrous females to screen for genetic quality. Much attention has focused on the potential for polyandrous females to accrue such benefits via a sexy- or good-sperm mechanism, whereby additive variation exists among males in sperm competitiveness. Likewise, attention has focused on an alternative model, in which offspring quality (in this context, the sperm competitiveness of sons) hinges on an interaction between parental haplotypes (genetic compatibility). Sperm competitiveness that is contingent on parental compatibility will exhibit nonadditive genetic variation. We tested these models in the Australian cricket, *Teleogryllus oceanicus*, using a design that allowed us to partition additive, nonadditive genetic, and parental variance for sperm competitiveness. We found an absence of additive and nonadditive genetic variance in this species, challenging the direct relevance of either model to the evolution of sperm competitiveness in particular, and polyandry in general. Instead, we found maternal effects that were possibly sex-linked or cytoplasmically linked. We also found effects of focal male age on sperm competitiveness, with small increments in age conferring more competitive sperm.

KEY WORDS: **Genetic compatibility, good sperm, sexy sperm, sperm competition, sperm offense.**

The reasons why the females of so many species mate multiply within a given reproductive bout (polyandry) remain something of an evolutionary enigma (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Stockley 2003; Simmons 2005). One certainty that arises from polyandry, however, is that it creates intense rivalry between males for fertilizations (Parker 1970). This rivalry typically continues after mating has taken place, in the form of sperm competition (Birkhead and Møller 1998; Simmons 2001b). Given that a male's fitness is determined largely by the number of eggs he can fertilize during his lifetime, sperm competition is expected to impose intense post-copulatory sexual selection on the sperm and accessory gland proteins (*Acps*) within the ejaculate. This expectation has been repeatedly corroborated, through empirical work that has documented strong selection on phenotypic variation in individual components of sperm quality (Radwan 1996; LaMunyon and Ward 1999; Gage and Morrow 2003; Gage et al. 2004; García-González and Simmons 2005b), in expression levels of *Acps* (Clark et al. 1995; Chapman 2001), and in the competitive ability of the ejaculate as a whole [hereon referred to as sperm competitiveness] (Simmons 2001b). It

follows that these traits are expected to respond to this selection, and evolve adaptively (Simmons 2001b; Evans and Simmons 2008).

Some of the most prominent models put forward to explain the evolution of polyandry center around the genetic benefits that females stand to gain by instigating postcopulatory sexual selection on the male ejaculate (Curtsinger 1991; Yasui 1997; Jennions and Petrie 2000; Simmons 2005; Evans and Simmons 2008). The "sexy-sperm" (Curtsinger 1991; Keller and Reeve 1995) and "good-sperm" (Yasui 1997) models (post-copulatory equivalents of the "Fisherian run-away" and "good genes" models of sexual selection) have received much attention (Simmons 2001b, 2005; Evans and Simmons 2008). Under both of these models, a male's fertilization success during sperm competition will be a product of his sperm competitiveness phenotype, and this phenotype will have an additive genetic component and therefore be heritable. In the case of the sexy-sperm model, polyandrous females who ensure that their ova are fertilized by males expressing high sperm competitiveness stand to produce sons that inherit this trait and have high relative fitness themselves (Curtsinger 1991). In the case of the good sperm model, sperm competitiveness is linked to general genetic viability, and thus polyandrous females that have their ova fertilized by males with competitive sperm will produce both sons and daughters that have higher relative fitness (Yasui 1997).

Although the sexy- or good-sperm models have received some support at the phenotypic level (i.e. in sperm competitiveness assays, some males consistently perform better than others [Evans and Rutstein 2008; Sherman et al. 2009], but see [Tregenza et al. 2009]), evidence garnered at the genetic level is equivocal. In particular, the assumptions that sperm competitiveness is heritable, and can respond adaptively to selection, are fundamental to the assessment of both models. These assumptions have recently come under renewed scrutiny (Dowling et al. 2007a, 2007b; Evans and Simmons 2008; Friberg and Dowling 2008; Zeh and Zeh 2008; Simmons and Moore 2009). Studies that have dissected the genetic architecture of individual ejaculate traits have generally found ample additive genetic variance for such traits (Simmons and Moore 2009). However, few studies have explored the quantitative genetics of sperm competitiveness per se, and those that have, have produced results that are inconsistent across and within taxa. Although "offensive" sperm competitiveness (male success at displacing resident sperm in a once-mated female, i.e., in the *P2* position) appears to harbor high levels of standing additive genetic variance in the Acarid mite *Rhizoglyphus robini* (Radwan 1998), the same trait seems to harbor no additive genetic variance in Australian field crickets *Teleogryllus oceanicus* (Simmons 2003). In one population of the fruitfly *Drosophila melanogaster*, Friberg et al. (2005) detected low levels of additive genetic variance (across haploid nuclear genomes) for both "defensive" (male success at resisting sperm displacement, i.e., in the *P1* position) and offensive sperm competitiveness, whereas Hughes (1997) found no additive gene action (across third chromosomes) for the same traits in another population. Thus, although individual ejaculate traits typically harbor reasonable amounts of additive genetic variance, and high heritabilities, sperm competitiveness per se is typically associated with low genetic additivity and low heritabilities (Simmons and Moore 2009). Furthermore, although these results for male fertility traits are concordant with the general principle in evolutionary genetics that the greater the contribution a given trait makes to "fitness," the lower its heritability (Mousseau and Roff 1987; Falconer and Mackay 1996), they are discordant with another general observation that the greater the contribution of a trait to fitness, the higher its coefficient of additive genetic variation and, hence, evolutionary potential (Houle 1992).

Other prominent models put forward to explain the evolution of polyandry center around "genetic compatibility," with the central premise being that genetic interactions between males and females are key contributors to offspring genetic quality. Genetic compatibility ideas come in two basic forms. First, polyandry might increase the opportunity for females to screen gametes of different males (i.e., at the postcopulatory stage) on the basis of their compatibility (Zeh and Zeh 1997; Jennions and Petrie 2000; Tregenza and Wedell 2000), using sperm competition in tandem with cryptic sperm-egg molecular recognition processes to ensure fertilization with best-matching sperm (Zeh and Zeh 1997). Under this scenario, the sperm competitiveness of any given male will not rest solely on the set of genes that he harbors, but will vary according to the genotypes of the females with which he copulates (i.e., male $\times$ female interactions) and the (haploid) genomes (of sperm) of the rival males who copulated with those same females (i.e., male $\times$ male, or male $\times$ male $\times$ female interactions). There is some support for this idea, with a number of studies showing that the outcome of sperm competition is nontransitive in some (Wilson et al. 1997; Zeh and Zeh 1997; Clark and Begun 1998; Clark et al. 2000; Clark 2002; Birkhead et al. 2004; Bjork et al. 2007; Dowling et al. 2007a), but not all (Lewis and Austad 1990; Radwan 1998; Arnqvist and Danielsson 1999; House and Simmons 2005; Evans and Rutstein 2008; Sherman et al. 2009), species.

Second, by inciting sperm competition, females might increase the chances that some of their offspring receive favorable allelic combinations. In quantitative genetic terms, the importance of these allelic interactions to the expression of any given trait will be revealed by the amount of nonadditive genetic variance underlying that trait (Neff and Pitcher 2005; Puurtinen et al. 2005). Nonadditive genetic variation encompasses interactions between alleles (inherited from each parent) at the same locus (dominance) and also between alleles at different loci

(epistasis). Although several studies have now demonstrated that such parental allelic interactions can be important contributors to early-life offspring performance and survival—i.e., traits determining juvenile fitness (Wedekind et al. 2001; Pitcher and Neff 2006; Evans et al. 2007; Ivy 2007; Pitcher and Neff 2007; Bilde et al. 2008; Dziminski et al. 2008; Rodríguez-Muñoz and Tregenza 2009), very little is known about the contribution of such parental interactions to fitness-related traits expressed by offspring of reproductive age—i.e., adult fitness (Bilde et al. 2008), and this holds true for traits tightly linked to adult male fitness, such as sperm competitiveness. The only previous study to screen for nonadditive genetic variance for sperm competitiveness indeed found such variation across third chromosomes in a population of *D. melanogaster* (Hughes 1997), which suggests that this form of genetic compatibility might be an important contributor to male fitness under sperm competition.

A previous quantitative genetic experiment (half-sibling analysis) in *T. oceanicus* revealed an absence of additive genetic variance for offensive sperm competitiveness, but a strong dam effect (Simmons 2003). The evolutionary significance of this dam effect is unclear because it incorporates unknown fractions of nonadditive genetic variance and variance attributable to maternal and other environmental effects. In this study, we reassess the genetic architecture of sperm competitiveness in *T. oceanicus*, by using a "full diallel" design that allows comprehensive partitioning of the additive, nonadditive genetic, and respective parental variances. In so doing, we provide (1) one of the few quantitative genetic tests of the potential for the genetic compatibility model to contribute to the evolution and maintenance of polyandry in general (Wedekind et al. 2001; Pitcher and Neff 2006; Evans et al. 2007; Ivy 2007; Pitcher and Neff 2007; Bilde et al. 2008; Dziminski et al. 2008; Rodríguez-Muñoz and Tregenza 2009), and (2) the first explicit quantitative genetic test of the role of genetic compatibility between parental genotypes in determining a son's subsequent reproductive performance under sperm competition.

Methods

STUDY POPULATION AND CONSTRUCTION OF INBRED LINES

Crickets used were the immediate descendents of a sample ($n >$ 100) of wild-type adults collected from a banana plantation in Carnarvon, north-west Western Australia, in 2006. Unless otherwise specified, all crickets were subsequently maintained in the laboratory at 27°C on a 12 h light:12 h dark photoperiod in 5 L plastic containers, with ad libitum access to dry cat food and water. Ten females were randomly sampled and used to found 10 separate isofemale lines, each of which was immediately subjected to five generations of full-sibling inbreeding. That is, each line was propagated by a single mating between a virgin daughter and her full-sibling brother for five sequential generations. At this point, the estimated inbreeding coefficient (F) of each inbred line was 0.672. During this inbreeding protocol, the offspring of each line were reared across two separate 5 L containers for most of their development, and sorted by sex (one box per sex) at their penultimate moult into adulthood.

DIALLEL CROSSES

The isofemale inbred lines were not very productive (a mean of only 13 pairs per line were produced at the fifth generation of inbreeding), and thus the resulting set of lines could only sustain an incomplete 8 × 8 full diallel reciprocal crossing scheme, performed in duplicate. Thus, the two weakest lines were discarded. For each of the remaining eight lines, a virgin adult female was mated once to a virgin male from one of the other seven lines, in all possible combinations. We omitted inbred combinations from the crossing scheme—that is, we did not cross females from a given inbred line to males from the same line. Each mated female was then housed in a plastic container (16 × 12 × 5 cm) for two weeks (or until death) and provided with food and a moist cotton pad (which was replaced after 7 days with a fresh pad) on which the female could oviposit. The emerging nymphs were transferred to 5 L containers with food and water. To mitigate potential density-dependent effects, we added two egg cartons to each container, and capped the maximum density of crickets at 30 per container.

We lost a number of cells from the diallel crossing scheme due to reproductive failure and mortality of the inbred parents involved in the crosses. This resulted in some crosses in the diallel not being represented at all, and others being represented by only one of the two replicates (Fig. 1). Roff and Sokolovska (2004) experienced similar problems when conducting diallel crosses in the sand cricket, *Gryllus firmus* (15 of 42 cells lost). These missing cells might affect the variance component estimates through a general loss of statistical power, or if the missing cells were distributed throughout the diallel in a nonrandom way. However, we note that unless the missing cells consistently involved lines with intermediate breeding values (an unlikely scenario because the weaker lines are likely to be the ones that failed to produce F_1 offspring), then any bias in the genetic variance estimates will be in a downward direction and, thus, our estimates can be taken as conservative.

MEASURING SPERM COMPETITIVENESS

F_1 offspring were reared to adulthood. Once offspring began to moult into adulthood, we checked each container three times per week to extract virgin adults. All F_1 adult males were placed individually in small holding containers (7 × 7 × 5 cm), with food and water, for nine to 13 days before being scored for sperm

	1B	4A	7C	8A	10C	3D	5D	9D
1B		7, 11	3, 0	0,0	10,10	7,9	1,0	7,0
4A	7,0		2,0	2,11	6,0	1,10	1,5	8,0
7C	7,0	10,0		9,10	11,9	2,1	5,3	4,8
8A	9,0	8,0	2,0		8,11	1,5	8,4	4,11
10C	5,0	0,0	0,0	0,0		1,0	0,0	0,0
3D	0,0	8,8	0,0	6,3	0,0		0,0	0,0
5D	0,0	9,2	0,0	0,0	0,0	0,0		0,0
9D	0,0	1,4	3,0	0,0	0,0	0,0	3,0	

Figure 1. The diallel crossing scheme used. Eight lines were crossed to each other in all combinations (with dams on the vertical and sires on the horizontal), barring the inbred combinations. Cells shaded gray indicate those crosses that were replicated and hatched cells represent crosses without replication. Blank cells represent combinations that failed to produce sons that reached adulthood. The number of F_1 sons subsequently scored (for sperm competitiveness) per replicate and cross are indicated within each cell. For example, the cross combination Dam 1B × Sire 4A was replicated, with seven F_1 sons from the first replicate, and 11 sons from the second replicate, scored for sperm competitiveness.

competitiveness. These became the focal males in the sperm competitiveness assays.

Wild-type crickets have black eyes (*be*). To measure sperm competitiveness, we used an autosomal recessive mutation encoding white eyes, which arose spontaneously in the laboratory (Simmons 2003). A population of white eye (*we*) crickets was established by backcrossing the white eye mutation to the wild-type genetic background and collecting the *we* offspring. The expression of the white eye allele follows a simple mode of Mendelian inheritance (homozygous recessive), which indicates that the general fertility of *we* males, and the viability of offspring sired by *we* males, does not differ from that of *be* males (Simmons et al. 2003).

Prior to the experiment, the size of the stock *we* population was increased by seeding each of 60 containers with 30 white-eye nymphs, staggered across time, such that adequate numbers of newly emerging *we* adults were available throughout the experiment. These *we* crickets were sorted and stored by sex at their penultimate moult, to ensure their virginity as adults. All *we* crickets used in the subsequent assays were either 10 or 12 day old adults.

Focal males were scored for their sperm competitiveness phenotype when in competition against one virgin *we* male. All focal males were scored in the *P2* position, and thus our measure indicates offensive sperm competitiveness. Specifically, a *we* virgin female was first mated to a *we* male, and the female then monitored closely to ensure that the *we* male's spermatophore remained attached to the female for 40 min. Twenty-four hours later, each once-mated *we* female was then presented with a focal *be* virgin male until she remated. Each *be* focal male was given one opportunity to mate. That is, focal males that failed to mate on their given assay date were excluded from the experiment. Again, we monitored closely each female, ensuring that the *be* male's spermatophore remained attached for 40 min. Each female was then provided with a cotton pad on which to oviposit. The nymphs produced from each double-mating were then collected 21 days later, and their eye color was scored. Sperm competitiveness of each focal male was measured as the proportion of *be* nymphs within the brood (mean brood size = 209, SE = 4.18, $n = 341$).

DATA ANALYSIS

We used the *bio model* described by Cockerham and Weir (Cockerham and Weir 1977) and Lynch and Walsh (1998) to estimate variance components. The model equation was

$$Y_{ijkl} = \mu + N_i + N_j + T_{ij} + M_j + P_i + K_{ij} + R_{k(ij)} + W_{l(k(ij))},$$

where Y_{ijkl} is the sperm competitiveness of the lth son from the kth replicate of the cross between sire i and dam j, and μ = mean sperm competitiveness of the population. N_i and N_j are the additive effects of the nuclear genes contributed by i and j, independent of sex; T_{ij} is the interaction of the haploid nuclear contributions; M_j is the maternal genetic and environmental effects of line j when used as dams; P_i is the paternal genetic and environmental effects of line i when used as sires; K_{ij} is the interaction between maternal and paternal effects; $R_{k(ij)}$ is the effect of kth replicate cross within the dam line × sire line combination and $W_{l(k(ij))}$ is the unique effect of individual l within a replicate cross (Fry 2004; Ivy 2007; Bilde et al. 2008).

We estimated these variances by Restricted Maximum Likelihood Analysis (REML) using the MIXED procedure in SAS version 9.1.3 (SAS Institute 2004), and the TYPE = LIN command to model the covariance between families as linear functions of the variances. The analysis was performed on a matrix of 56 between-line crosses (excluding the within-line crosses). We tested the one-sided hypotheses that parameter estimates are larger than 0 with likelihood ratio tests, by comparing models in which the parameter of interest was set to 0 with a model where all parameters were allowed to assume nonnegative values (see Fry 2004 for details of this model and associated example script). Although the analyses we present were performed on sperm competitiveness values that were not transformed (Houle 1992), we note that an analysis on arcsine transformed sperm competitiveness values yielded qualitatively identical results, in terms of the significance tests. In addition to the script outlined in Fry (2004), we included a statement that separates the variance among males within replicate crosses from the variance among replicate crosses. We also included the age of the F_1 focal males as a fixed factor in the model.

We then estimated the causal components of the observational variance components using the same methods outlined in Fry (2004) and Bilde et al. (2008):

(1) σ^2_n: nuclear additive variance. See Bilde et al. (2008) for calculations. Briefly, if epistasis is assumed to be small, then the additive variance, V_A, can be calculated as $2\sigma^2_n/F$.
(2) σ^2_t: nuclear interaction variance. Assuming low epistatic variance, then the dominance variance, V_D, can be calculated as σ^2_t/F^2.
(3) σ^2_m: maternal effect variance V_M, including both maternal genotype and environment effects, and possible interactions between maternal nuclear and maternal extra-nuclear effects.
(4) σ^2_p: paternal effect variance V_P, including both paternal genotype and environment effects, and possible interactions between paternal nuclear and paternal extra-nuclear effects.
(5) σ^2_k: interaction variance, V_K, of paternal and maternal effects, and of nuclear and extra-nuclear effects.
(6) σ^2_{rep}: variance among replicate crosses within-line combinations.
(7) σ^2_w: variance among males within-replicate crosses.

Coefficients of genetic variation (CV) for each variance component estimate were calculated by scaling the causal variance components by the trait mean; e.g.

$CV_A = 100(\sqrt{V_A})$/trait mean (Houle 1992).

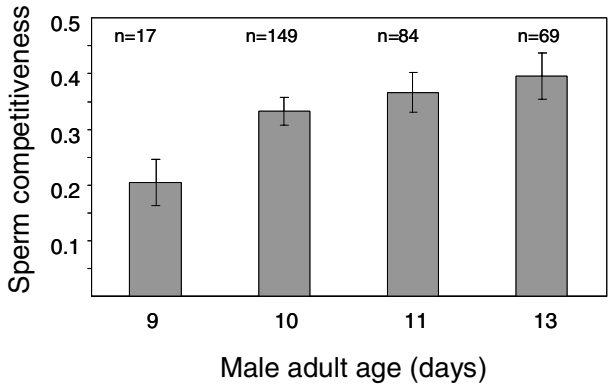

Figure 2. Mean (±1 SE) sperm competitiveness (P2) for focal males of each age class (9, 10, 11, and 13 days following collection as virgins).

Results

Mean sperm competitiveness across all focal males was 0.35 (n = 323, SE = 0.018). We detected no additive genetic variance for sperm competitiveness, and no genetic variance attributable to the interaction between nuclear haploid genomes, which would have indicated underlying dominance or epistatic gene action (Table 1).

We did, however, detect a significant maternal effect on the sperm competitiveness of the F_1 sons (Table 1), which accounted for about 7% of the phenotypic variation in this trait (and at least 2.3% based on the 95% Confidence Limits, Lower 0.002408, Upper 0.06798). This maternal effect encompasses both genetic (e.g., sex-linked and cytoplasmic/mitochondrial genetic) and environmental effects (e.g., common environments shared by all the mothers of a given line). Paternal effects on sperm competitiveness were much weaker (Table 1) and not statistically significant. There were no nonreciprocal interaction effects (as indicated by σ^2_k), and no variance was detected among replicate crosses (σ^2_{rep}).

Older focal males exhibited increased sperm competitiveness ($F_{3,264} = 2.72$, $P = 0.045$, Fig. 2).

Discussion

We detected no additive variance for offensive sperm competitiveness in *T. oceanicus*. This result confirms an earlier finding by Simmons (2003), thereby constraining the scope for a direct sexy- or good-sperm process in this species. Furthermore, we did not detect any nonadditive genetic variance for sperm competitiveness, which suggests that genetic compatibility between parental haplotypes makes no contribution to the subsequent fertilization success of sons during sperm competition.

A critical assumption of the sexy- or good-sperm models is that sperm competitiveness is associated with a genetic architecture that consists, in part, of additive genetic variance.

Table 1. Variance component estimates and coefficients of variation (CV). Note that the Estimation of V_D assumes little or no epistasis. *P* values are based on likelihood ratio tests (see Methods). Percent refers to the percentage of total variance explained by each parameter.

Observational	Causal	Estimate	SE	Percent	*P*	CV
σ^2_n	V_A	0	-	0	-	-
σ^2_t	V_D	0	-	0	-	-
σ^2_m	V_M	0.006971	0.005184	6.76599	0.0024	23.69832
σ^2_p	V_P	0.000609	0.001859	0.59109	0.655	7.092134
σ^2_k	V_K	0	-	0	-	-
σ^2_{rep}	V_{REP}	0	-		-	-
σ^2_w	V_E	0.09545	0.007715	92.64292		
	Total	0.10303	-			

Additive genetic variance is known to underlie sperm viability (the proportion of sperm alive in an ejaculate) in *T. oceanicus* (Simmons and Roberts 2005). Additive genetic variance also exists for the size of the male accessory glands (Simmons 2003), which produce the *Acps* likely to play a key role in deciding the outcomes of sperm competition (Chapman 2001). Furthermore, a male's sperm viability has previously been shown to correlate with his sperm competitiveness in this species (García-González and Simmons 2005b), although the correlations presented were phenotypic and therefore do not suggest the presence of an underlying genetic correlation between these two traits. For instance, the correlations in that study might have been influenced by phenotypic variation in a third trait. In light of previous findings, it is worthwhile addressing why additive genetic variance for individual ejaculate traits does not translate into additive variance for sperm competitiveness per se.

The answer to this question is unclear in *T. oceanicus*, but various factors may be involved. First, it has become increasingly clear that a male's sperm competitiveness phenotype can be contingent on the genotypes of interacting competitor males and females (Wilson et al. 1997; Clark and Begun 1998; Clark et al. 2000; Clark 2002; Bjork et al. 2007; Dowling et al. 2007a), and such genetic nontransitivity of sperm competitiveness will undermine the detection of additive variation for this trait (García-González 2008). It has yet to be tested whether sperm competitiveness exhibits genetic nontransitivity in *T. oceanicus*.

Second, the presence of negative genetic correlations between individual ejaculate components that are important in sperm competition, could contribute to a lack of genetic additivity for sperm competitiveness. In such a scenario, selection for increased expression of one trait will result in decreased expression in a correlated trait (e.g., some genotypes might exhibit high sperm motility and low sperm viability whereas others exhibit low motility and high viability). Thus, the effects of each ejaculate trait on a male's overall sperm competitiveness might ultimately cancel each other out, or at least result in a dilution in additive genetic variance for sperm competitiveness. Such negative genetic correlations appear to be common between ejaculate traits (Pitnick 1996; Levitan 2000; Moore et al. 2004; Schulte-Hostedde and Millar 2004; Dowling et al. 2007b).

Third, we envisage that genotype by environment interactions for ejaculate components might stand in the way of there being additive genetic variance for sperm competitiveness. For instance, ejaculate traits that indicate performance (e.g., motility and viability) are usually measured in vitro (e.g., in saline solution), and thus in the one environment. However, it is conceivable that the expression of these ejaculate traits will vary across environments, for instance across the reproductive tracts of different females. In support of this contention, Møller et al. (2008) measured various aspects of sperm quality in barn swallows, *Hirundo rustica*, in neutral medium and medium containing fluid derived from the reproductive tracts of different females. Their results not only indicated sperm quality differed when measured in neutral versus female-derived media, but also suggested that the expression of certain sperm components varied according to the quality of females from which the fluid was extracted (Møller et al. 2008).

The criterion of additive genetic variance for offensive sperm competitiveness is fundamental to the validity of the good- and sexy-sperm models of sexual selection. However, this criterion is not necessarily exclusive to these models. In this regard, several studies have now reported negative correlations between male offensive sperm competitiveness and female fitness in *D. melanogaster* (Friberg et al. 2005) and *Callosobruchus maculatus* seed beetles (Bilde et al. 2009; Hotzy and Arnqvist 2009), and similar correlations are known to exist between defensive sperm competitiveness and components of female fitness in *D. melanogaster* (Civetta and Clark 2000; Wigby and Chapman 2005; Wigby et al. 2009). Such correlations indicate that postcopulatory sexual selection can favor male sexually antagonistic adaptations that enhance sperm competitiveness while harming females. These correlations are at odds with the idea that females will mate multiply to acquire genetic benefits, because the fitness costs of mating are likely to outweigh any indirect genetic benefits gained (Arnqvist and Rowe 2005). Yet, under this model of the evolution of sperm competitiveness, we would also expect there to be additive genetic variance underlying offensive sperm competitiveness. The fact that we did not find such genetic variance would therefore also seemingly undermine the relevance of sexual conflict to the evolution of sperm competitiveness in *T. oceanicus*.

We detected a sizeable maternal effect on sperm competitiveness, and this effect was much larger than any such paternal effect. This result is striking because it is the exact opposite pattern to that expected under a sexy- or good-sperm scenario. This maternal effect may either be environmental or genetic (e.g., X chromosome- or cytoplasmically linked). Environmental maternal effects will be more prevalent, and stronger, on traits related to the performance of offspring early in life (e.g., egg size and hatching rates). However, such effects can also linger into offspring adulthood (Roff 2002), even to the extent that they might influence aspects of sperm performance (Dowling et al. 2007b; Gay et al. 2009). We mitigated the possibility that the observed maternal effect was driven by line-specific rearing environments, by raising each parental line across two replicate containers for the majority of their development (see Methods). Although all of the females of any given parental line did share some time (final two moults and first 10 days of adulthood) within the same 5 L container before being used in the diallel crosses, all lines were maintained at low densities under controlled and nonstressful (ad lib food and water) conditions. We also point out that

we found no container-specific environmental effect on F_1 sperm competitiveness (as indicated by zero variance for V_{REP}), and that the F_1 offspring were reared under very similar conditions to those of the parental lines. However, although the F_1 sons assayed for sperm competitiveness were not inbred, their mothers were. This might well have increased the likelihood of there being environmental maternal effects, in the event that some maternal isofemale inbred lines suffered higher levels of inbreeding depression than others and were thus in poorer general condition. Two points, however, would suggest that inbreeding depression was not responsible for the maternal effects observed in this study. First, although females from the isofemale inbred lines appeared to exhibit lower fecundity than those in the outbred population from which they were sourced, five generations of full-siblings inbreeding should have been enough to have purged many of the deleterious recessive alleles segregating within the isofemale inbred lines. Second, a previous half-sibling quantitative genetic analysis of sperm competitiveness in *T. oceanicus* also revealed strong dam effects (Simmons 2003), and given the absence of nonadditive genetic effects in the present study, we can now conclude that these dam effects were attributable to maternal effects. Notably, all mothers in that half-sibling breeding design were sampled directly from a large and outbred wild population. Therefore, the maternal effects in Simmons (2003) cannot be attributed to inbreeding depression, which lends support to the argument that the maternal effects found in our study are not driven by inbreeding effects.

Although we cannot completely dismiss the possibility that the maternal effects found here were environmentally induced, it is nevertheless worth addressing whether the maternal effects might have been genetic in origin. Several studies have now demonstrated maternal genetic effects on individual components of sperm morphology (Ward 2000; Morrow and Gage 2001; Birkhead et al. 2005; Dowling et al. 2007b) or sperm quality (Dowling et al. 2007b), with the respective authors suggesting that these effects are either encoded by genes on the X chromosome (Ward 2000; Morrow and Gage 2001) or in the mitochondrial genome (Dowling et al. 2007b). However, such maternal genetic effects have never been shown to translate into absolute effects on male fertilization success under sperm competition (Dowling et al. 2007a, 2008; Friberg and Dowling 2008). Our results here are the first to support, although tentatively, the idea that maternally transmitted genes (i.e., on the X and in the cytoplasm) can encode consistent differences in the sperm competitiveness of sons.

Sperm competitiveness increased with the age of the focal males. Other studies to have found effects of male age on sperm competitiveness have typically examined effects over very distinct age classes (Service and Fales 1993; Schäfer and Uhl 2002; Jones and Elgar 2004; Radwan et al. 2005). Our result is particularly notable because all males were assayed within a four-day window (mean of 9–13 days of age) early in life (males remain fertile up to 30 days of age under laboratory conditions, García-González and Simmons 2005b). Thus, our results show that the sperm competitiveness phenotype in *T. oceanicus* is highly sensitive to subtle variation in age. Although it is unclear whether sperm competitiveness plateaus at some intermediate age, or exhibits senescence in late life, if age and sperm competitiveness covary with some other measure of viability (e.g., rate of de novo germline mutations), then polyandry will allow females to tap into this age-related variance in sperm competitiveness to bias fertilizations toward the most viable males (Radwan 2003).

The force of selection decreases with age and, thus, mutations with effects restricted to late-life will equilibrate at higher levels under mutation–selection balance than mutations expressed earlier in life (Charlesworth 2000). Consequently, the amount of additive, dominance, and general genetic variance underlying any given fitness trait is predicted to increase with age (Charlesworth and Hughes 1996). This prediction has been tested on several occasions, with mixed results (Promislow and Tatar 1998). Notably, Kosuda (1985) found an age-related increase in the Coefficient of Variation for male mating ability (proportion of females a male inseminated under noncompetitive conditions) among lines of *D. melanogaster* that were homozygous for different second chromosomes, and Hughes (1995) found increases in additive genetic variance for male mating ability with increasing age. In this study, we measured offensive sperm competitiveness relatively early in life (Day 9 to 13 of a mean 45-day life span under laboratory conditions, estimated from females, L. W. Simmons, unpubl. data). It is therefore possible that we might have detected genetic variance (both additive and nonadditive) for this trait had we measured it at a later-life stage, and this point seems reinforced by the above observation that sperm competitiveness is indeed sensitive to variation in age. However, we note that the mean life span of crickets in the wild (around 12–14 days in males) is substantially lower than in the laboratory (Simmons and Zuk 1994), and therefore our measure of sperm competitiveness between day 9 and 13 is likely to be the most biologically relevant life stage at which to have measured this trait.

One of the most prominent arguments put forward to explain the evolution of polyandry is that it allows females to screen male genetic quality at the postcopulatory stage, by inciting sperm competition and cryptic female choice (Zeh and Zeh 1997; Jennions and Petrie 2000; Simmons 2005). Our results are generally unsupportive for this idea, both for the sexy- or good- and compatible genes processes, because we were unable to detect any consistent genetic differences (other than possible maternal genetic) in the competitiveness of sperm, and thus resulting fitness, of males within the population. Furthermore, although our results do not preclude the possibility that good or compatible genes might underlie differences in other fitness components (e.g., adult female fitness) in *T. oceanicus*, they do suggest that females cannot screen

for such genetic benefits at the postcopulatory stage via sperm competition. Rather, evidence is mounting to show that female *T. oceanicus* who mate multiply, with different males, can enhance the viability of their offspring via direct benefits associated with male seminal products (Simmons 2001a; García-González and Simmons 2005a, 2007). This is consistent with Arnqvist and Nilsson's (2000) conclusion, based on a meta-analysis across insect taxa, that the evolutionary maintenance of polyandry can be explained fully in terms of direct benefits.

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