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Original Article

A test of the sexy-sperm and good-sperm hypotheses for the evolution of polyandry

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The sexy-sperm hypothesis proposes that polyandrous females will have a selective advantage over monandrous females as their sons will be sired by males with competitively superior sperm and will inherit this trait. Although the good-sperm hypothesis also predicts that offspring will be sired by males with competitively superior sperm, it additionally assumes a positive correlation between offspring sperm quality and the general viability of both sons and daughters. We examined the potential for sexy-sperm and good-sperm processes by exploring the effect of polyandry on offspring sperm quality and immunocompetence in the field cricket, *Teleogryllus oceanicus*, a species in which sperm viability predicts sperm competitive ability. In the parental generation, females were mated monandrously or polyandrously, where each female received 3 matings. We reared the resultant offspring through to maturity and assayed approximately 10 offspring per family for both sperm viability and immunity. We found that male offspring from polyandrous mothers had higher sperm viability, but that this was not phenotypically associated with male or female offspring condition, as measured by either immune function or ability to survive a bacterial infection. Thus, our data are consistent with a sexy-sperm, but not a good-sperm model in explaining the evolution of polyandry in this species.

Key words: **ecological immunology, field cricket, indirect selection, offspring fitness, postcopulatory female choice, sexual selection, sperm viability.**

INTRODUCTION

The factors that shape the evolution of female multiple mating, or polyandry, remain a significant question for evolutionary biologists (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Kvarnemo and Simmons 2013; Parker and Birkhead 2013). Although the benefits of polyandry have been well established in mating systems in which females derive direct benefits from mating (Arnqvist and Nilsson 2000), the maintenance of polyandry in species where females gain no immediate advantage from mating are less clear (Slatyer et al. 2012). Several hypotheses exist that propose mechanisms for indirect benefits to females of multiple mating, although these need not be mutually exclusive of direct benefits. Analogous to the well-established precopulatory good-genes and sexy-sons models of female choice, the good-sperm and sexy-sperm hypotheses suggest that when females are polyandrous, there is selection for male traits that increase sperm competitiveness (Curtsinger 1991; Keller and Reeve 1995; Yasui 1997). Specifically, the sexy-sperm hypothesis proposes that polyandrous females will have a selective advantage over monandrous females as their sons will be sired by males with competitively superior sperm and will inherit this trait (Curtsinger 1991; Keller and Reeve 1995). The good-sperm hypothesis also predicts that offspring will be sired by males with competitively superior sperm, however, it additionally posits a positive correlation between a male's sperm competitiveness and the general viability of both sons and daughters (Yasui 1997), which can be measured using traits such as survival or immunocompetence. It should be noted that the sexy- and good-sperm process, despite their names, refer to any traits, including non-sperm components of the ejaculate, that might influence fertilization success, and that female choice is manifest directly via the morphology and physiology of their reproductive tract that might affect fertilization success or indirectly via a female's propensity to re-mate and so incite sperm competition among males (Keller and Reeve 1995; Yasui 1997). A key assumption of both good- and sexy-sperm models is that the paternal sperm-competitive traits that incur increased fertilization success are heritable.

Despite being of great theoretical interest (Curtsinger 1991; Keller and Reeve 1995; Yasui 1997; Evans and Simmons 2008), there remains relatively little direct evidence for either good- or sexy-sperm processes (but see Hosken et al. 2003; García-González and Simmons 2007b). In general, there is evidence for the potential of both good- and sexy-sperm processes in the form of additive genetic variance and repeatability of individual sperm traits (for a

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review, see Evans and Simmons 2008; Simmons and Moore 2009), although rarely for sperm competitiveness per se (Simmons 2003; Dowling et al. 2010). There is some experimental evidence, albeit equivocal, for sexually selected sperm processes in the red flour beetle, *Tribolium castaneum*, in which polyandrous mothers produced sons that were more successful in defensive, but not offensive sperm competition in wild-type, but not black color morphs (Bernasconi and Keller 2001). There is also some evidence for good-sperm processes. For example, in the yellow dung fly, *Scatophaga stercoraria*, highly sperm-competitive males produced offspring that developed at a faster rate, a trait likely to be important for survival in this species (Hosken et al. 2003). Evidence of good-sperm processes also comes from the dung beetle *Onthophagus taurus*. Males that produce relatively shorter sperm have a higher body condition, these traits are genetically correlated (Simmons and Kotiaho 2002), and shorter sperm have a higher fertilizing capacity, especially in larger female sperm stores (García-González and Simmons 2007b). Finally, in the antechinus *Antechinus stuartii*, males that gain high paternity under sperm competition sire offspring that are more likely to survive (Fisher et al. 2006). Together, these studies demonstrate the potential for female postcopulatory preferences to bias fertilization toward males of intrinsicaly higher quality.

There are several potential reasons for the paucity of experimental evidence for sexually selected sperm models. First, several theoretical studies have highlighted the potential for the genetic architecture of sperm traits to constrain the evolution of good- and sexy-sperm processes (Pizzari and Birkhead 2002; Gemmell et al. 2004). In particular, these studies highlight the potential influence of maternal effects, such as X-linkage and cytoplasmic genes, on sperm traits that preclude the father-to-son heritability that underlies sexually selected sperm models (for a review, see Evans and Simmons 2008). Second, several quantitative genetic studies have failed to find support for the sexually selected sperm hypothesis, based on the absence of additive genetic variance for sperm competitiveness (Simmons 2003; Dowling et al. 2010). Genetic variance in sperm competitiveness is typically assessed by estimating the proportion of offspring sired by the second of 2 males to mate (P_2). Yet, empirical studies have highlighted the importance of male × male × female genotype interactions in determining competitive fertilization success (Bjork et al. 2007), which is confirmed in mathematical models that show fertilization success to be an imperfect index of male sperm competitiveness, with the stochastic nature of male–male–female interactions underestimating the additive genetic variance in sperm competitive ability (García-González 2008). Ultimately, however, despite the absence of observable additive genetic variance for sperm competitiveness among several studied species, the genetic architecture required for sexually selected sperm processes appears to exist: Individual sperm traits typically exhibit strong additive genetic variance and are often autosomal or Y-linked in nature (Simmons and Moore 2009); sperm competitiveness is often repeatable (House and Simmons 2005); and sperm and sperm-related traits can respond rapidly to selection (Hosken and Ward 2001; Simmons and García-González 2008; Nandy et al. 2013).

In establishing precopulatory good-genes or postcopulatory good-sperm processes, a number of offspring viability indicators are used to demonstrate relationships between father's attractiveness/sperm competitiveness and offspring fitness, including survival, growth, developmental duration, condition, and immunocompetence. A recent meta-analysis of empirical tests of good-genes models demonstrated an association between male attractiveness and immunocompetence and condition, but not other life-history traits (survival, growth, or reproduction) (Prokop et al. 2012). Thus, immunocompetence may be a valuable indicator of offspring viability for good-genes and good-sperm models of female choice. Immunocompetence in invertebrate models is typically assayed *in vitro* by measuring 2 key immune parameters: the phenoloxidase (PO) cascade and lytic activity. PO is an enzyme released in the hemolymph following the detection of a foreign body. Its activation results in the melanization and death of the pathogen (Sugumaran 2002). In some species, PO activity correlates with an organism's ability to resist pathogens and parasites (Wilson et al. 2001; Rantala and Roff 2007) and is also involved in wound healing (Nam et al. 2012). Lytic activity is the main defense against bacteria and is measured as the ability to disrupt the structural integrity of invading pathogens. Lytic activity is measured by quantifying the ability of the hemolymph to destroy bacterial cells. As neither PO nor lysozyme activity necessarily correlates with an individual's resistance to disease (Adamo 2004a), immunocompetence may also be measured *in vivo* by measuring the ability of an animal to survive a quantified parasitic, bacterial, or viral infection.

The highly polyandrous Australian field cricket, *Teleogryllus oceanicus*, is an excellent species with which to test sexy-sperm and good-sperm models for several reasons. First, a significant predictor of sperm competitiveness has already been established. Sperm viability, measured as the proportion of live sperm in the ejaculate, is an accurate predictor of male paternity success in a competitive context; the relative paternity success of 2 males conforms to a fair raffle whereby the viability of each male's sperm directly affects their relative share in paternity (García-González and Simmons 2005). Spermatophores can also be removed directly from the male's subgenital pouch and sperm viability determined without any potential for female interaction effects. Second, there is significant additive genetic variance for sperm viability in this species (Simmons and Roberts 2005). There is also additive genetic variance for male accessory gland size (Simmons 2003), which is involved in the production of seminal fluid proteins that are known to affect sperm viability (Simmons and Beveridge 2011). Thus, the genetic variance required for good and sexy-sperm processes to operate appears to exist. Finally, females mount males in this species, so that polyandry does not occur via forced copulations.

In order to test sexy-sperm and good-sperm processes, we adopted the classic protocol of Tregenza and Wedell (2002). We mated virgin females monandrously or polyandrously for a total of 3 copulations. Male mating experience was controlled across treatments, to exclude differences in total ejaculate size received by the female that may arise in relation to male mating frequency. Offspring from each family were reared to sexual maturity and sperm viability and 3 immunocompetence traits were measured. In line with both sexy- and good-sperm models, we predicted that sons of polyandrous mothers should have higher sperm viability. In line with good-sperm models, we also predicted that polyandrous mothers should have offspring with superior immune function and ability to survive infection.

METHODS

Experimental animals were derived from a large outbred laboratory stock population that was both initiated from and supplemented annually with individuals wild-caught in Carnarvon, Western Australia. The population is propagated by hundreds of adults per generation via mass matings. Individuals were housed

in a constant temperature room at 25 °C on a 12 h light: 12 h dark photoperiod.

Parental generation

Penultimate instar stock females were individually isolated in plastic containers (7 × 7 × 5 cm) and supplied with a water feeder (a vial filled with water and plugged with cotton wool) and ad libitum dried cat chow (26% protein and 8% carbohydrate). Upon adult emergence, females were weighed and assigned randomly to either a monandrous or polyandrous mating treatment. In the monandrous treatment (n = 38), females were mated 3 times with the same randomly selected stock-reared male within an 8-h period. In the polyandrous treatment (n = 36), females were mated once to 3 randomly selected males, also within 8 h. To ensure male mating history was comparable between treatments, "polyandrous" females mated first with a virgin male, next with a once-mated male, and finally with a twice-mated male. Females that did not complete all 3 matings in the time period were excluded. Thrice-mated females were returned to their containers and given wet cotton wool on which to oviposit.

Offspring generation

After 2 weeks, the cotton wool pads were removed and the eggs incubated until the juveniles hatched. Randomly selected first-instar nymphs were group housed (25 nymphs in four 5-l containers) with ad libitum access to cat chow and a water feeder. When the juveniles could be sexed, individuals were re-distributed to 2 single sex containers, each with 25 individuals. Containers were checked daily for newly emerged adults. Approximately 10 (range: 3–18) newly emerged adult males and females were removed from the family containers and allocated alternatively to one of 2 experiments. In one experiment, both males and females had their immune function tested and males additionally were assessed for their sperm viability. In the second experiment, the ability of males and females to survive a live bacterial challenge was assessed. Prior to experiments, adults were housed in plastic containers with ad libitum cat chow and water (as for the parental generation). Age at adult emergence and adult weight were recorded. All experiments were conducted on individuals 12–13 days following adult emergence.

Sperm viability assays

Males (monandrous parental background, n = 195; polyandrous parental background, n = 188) were assessed for immune function and sperm viability. A spermatophore was removed from the male's genital pouch, and the viability of the sperm was assessed using a Live:Dead Sperm Viability Kit (Molecular Probes, Eugene, OR). The kit differentially dyes viable (green) and dead (red) sperm in the ejaculate, allowing an estimate of the proportion of sperm viable. Sperm viability in *T. oceanicus* is a predictor of competitive male fertilization success (García-González and Simmons 2005). The spermatophore was cut at the neck and the sperm allowed to disperse into 20 µl of Beadle saline (128.3 mM NaCl, 4.7 mM KCl, 23 mM $CaCl_2$) on a glass slide. A 5 µl subsample of this solution was mixed gently on a glass slide with 5 µl of a 1:50 dilution of 1 mM SYBR-14 and incubated in the dark for 5 min. Next, 2 µl of propidium iodide was mixed in, and the solution incubated for another 5 min. A cover slip was placed over the solution and the sperm counted using a fluorescence optical filter at ×200 magnification. The color (viability) of the first 500 sperm observed from several randomly selected fields on the slide was determined. Approximately 10% of the sperm sampled can be doubly stained (Dowling and Simmons 2012), these were considered "dead." Thus, the viability of the sample was calculated as the proportion of green sperm in the total number of sperm (green, red, and doubly stained). Sperm counting was conducted blind to treatment, to reduce observer bias. Twenty-eight males did not produce a spermatophore after the 2-day testing period and were excluded from all further sperm and immune analyses (monandrous, n = 13; polyandrous, n = 15).

Immune assays

Hemolymph was extracted from females (monandrous, n = 208; polyandrous, n = 215) and from the males that had their sperm viability assayed (monandrous, n = 182; polyandrous, n = 173). Twenty microliters of hemolymph were collected after decapitating crickets on a piece of Parafilm. For PO assays, 2.5 µl was first added to an Eppendorf containing 122.5 µl of phosphate-buffered saline (PBS) before freezing at −80 °C. For lytic assays, 4 µl was added to 20 µl ice-cold PBS until analysis.

For the lytic assay, 10 µl of the diluted hemolymph sample was placed into a 96-well microtiter plate. To the sample, 10 µl of 1 mM sodium azide (Sigma Aldrich, New South Wales, Australia) to inhibit PO activity and 80 µl of a 3 mg/ml solution of *Micrococcus luteus* (Sigma Aldrich) in PBS were added. Each hemolymph sample was replicated twice. The plate was incubated at 33 °C for 2 h and then the absorbance measured at 492 nm in a microplate reader (M5 Spectramax; Molecular Devices, Sunnyvale, CA). Lytic activity was calculated as the difference in absorbance between the hemolymph samples and control samples (wells that differed only in containing PBS instead of diluted hemolymph).

For PO assays, 45 µl of the diluted hemolymph sample (1:50 hemolymph:PBS) were placed into a 96-well microtiter plate. To each sample, 45 µl of chymotrypsin (2.22 mg/ml in PBS) (C4129; Sigma Aldrich) were added and incubated at 25 °C for 20 min. After incubation, 90 µl of 9 mM dopamine hydrochloride (H8502; Sigma Aldrich) were added and the absorbance was measured at 492 nm every minute for 15 min (at 25 °C) using a microplate reader (M5 Spectramax; Molecular Devices). This period was determined previously to be in the linear phase of the reaction.

Immune challenge

In a separate experiment, male (monandrous, n = 183; polyandrous, n = 190) and female (monandrous, n = 204; polyandrous, n = 211) resistance to bacterial infection was assayed by injecting adults with a LD_{50} dose of *Serratia marcescens* (Dowling and Simmons 2012). Ten microliters of live *S. marcescens* which had been grown overnight at 37 °C in nutrient broth and diluted to a concentration of 0.675 million cells/10 ml (determined using a Spectramax plate reader) was injected into the body cavity of adult individuals. The survival of each individual was monitored over 4 days.

Statistical analyses

Data relating to immune activity and sperm viability were analyzed using linear mixed-effect models fit by restricted maximum likelihood (REML) with family identity as a random factor (JMP version 9.0.0; SAS Institute Inc., Cary, NC). LD_{50} mortality data were analyzed with mixed-effect survival analysis, with both family and bacteria batch (to account for potential variation in bacteria concentration) as random factors (Therneau 2012; R Core Team 2013). For sperm viability and immune analyses, 2 males were excluded from analyses as they did not have their weight recorded, and a further 2 males were excluded

as they produced an ejaculate with no sperm. In some cases, it was not possible to measure both PO and lytic activity for the same individual, due to insufficient hemolymph or experimenter error.

RESULTS

Sperm viability

The proportion of live sperm in a male's ejaculate (arcsine transformed) was higher for offspring of polyandrous compared with monandrous mothers (monandrous, n = 179; polyandrous, n = 172; $F_{1,67}$ = 79.12, P = 0.0001; Figure 1) and for heavier males ($F_{1,323}$ = 7.18, P = 0.008). An interaction between parental mating treatment and the weight of the male was not significant and was not included in the final model ($F_{1,320}$ = 0.12, P = 0.73). Family identity did not affect sperm viability (variance: 95% confidence interval [CI] = −0.001 to 0.001).

Male immunity

Male PO activity (cube root transformed) was not affected by the parental mating background (monandrous, n = 175; polyandrous, n = 161; $F_{1,69}$ = 0.09, P = 0.77; Figure 2a) or by the weight of the individual ($F_{1,331}$ = 0.00, P = 1.00). An interaction between parental mating treatment and the weight of the male was not significant and was not included in the final model ($F_{1,331}$ = 1.53, P = 0.22). Family identity accounted for 13.70% of the observed variance in PO activity (variance: 95% CI = 0.013–0.125).

Male lytic activity was not affected by the parental mating background (monandrous, n = 173; polyandrous, n = 162; $F_{1,58}$ = 0.31, P = 0.58; Figure 2b) or by the weight of the individual ($F_{1,324}$ = 1.29, P = 0.26). An interaction between parental mating treatment and the weight of the male was not significant and was not included in the final model ($F_{1,320}$ = 0.66, P = 0.42). Family identity did not affect lytic activity (variance: 95% CI = −0.001 to 0.005).

Female immunity

Female PO activity was not affected by the parental mating background (monandrous, n = 203; polyandrous, n = 210; $F_{1,56}$ = 0.31, P = 0.60) or the weight of the individual ($F_{1,380}$ = 3.26, P = 0.07; Figure 2a). An interaction between parental mating treatment and the weight of the female was not significant and was not included in the final model ($F_{1,378}$ = 0.15, P = 0.69). Family identity did not affect PO activity (variance: 95% CI = −15.095 to 15.331).

Female lytic activity was not affected by the parental mating background (monandrous, n = 202; polyandrous, n = 204; $F_{1,58}$ = 0.31, P = 0.58) or by the weight of the individual ($F_{1,324}$ = 1.29, P = 0.26; Figure 2b). An interaction between parental mating treatment and the weight of the female was not significant and was not included in the final model ($F_{1,401}$ = 0.19, P = 0.66). Family identity accounted for 14.05% of the observed variance in lytic activity (variance: 95% CI = 0.001–0.006).

LD_{50}

The proportion of individuals that survived the 4-day testing period was higher for males (z = −3.11, P = 0.002; Figure 3) and for heavier individuals (z = −2.86, P = 0.004), but was not affected by the parental mating treatment (z = −1.49, P = 0.14). An interaction between parental mating treatment and the sex of the individual was not significant and was removed from the model (z = 0.05, P = 0.96).

DISCUSSION

Polyandrous *T. oceanicus* females produced sons with higher sperm viability, a trait that predicts male sperm competitiveness in this

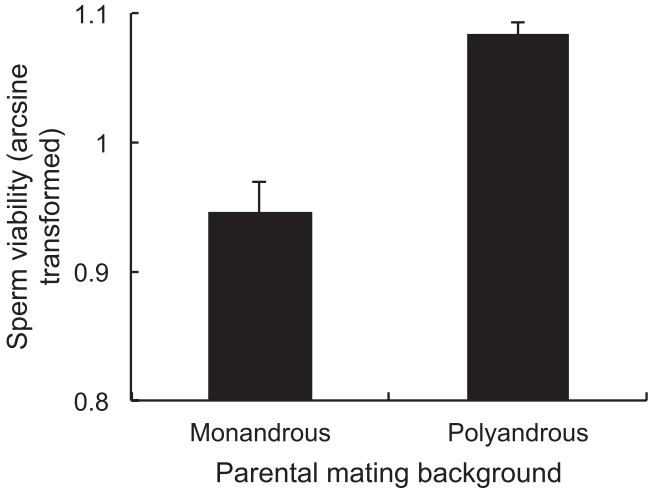

Figure 1
The proportion of live sperm (arcsine transformed) in the ejaculates of males from either monandrous or polyandrous parental mating backgrounds.

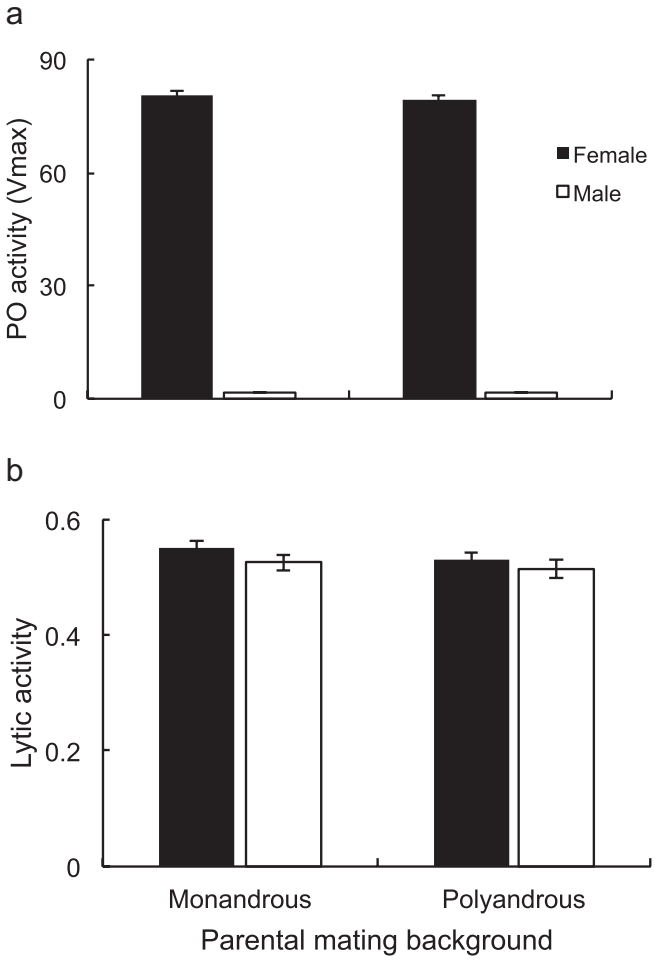

Figure 2
Mean ± standard error for (a) PO and (b) lytic activity for offspring from monandrous or polyandrous parental mating backgrounds.

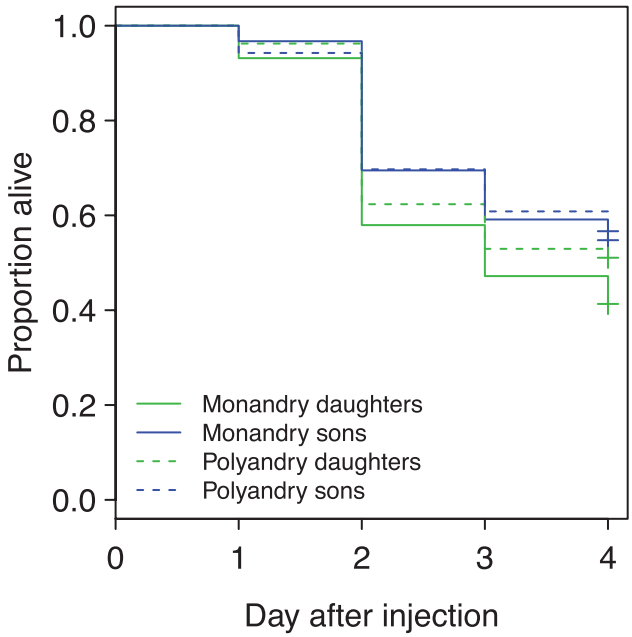

Figure 3
The proportion of sons and daughters from monandrous or polyandrous parental backgrounds that survived following a bacterial infection.

species. However, polyandry did not lead to increased immunocompetence in standing immunity or in an offspring's ability to survive a live bacterial immune challenge. The relationship between increased sperm competitiveness and offspring viability is central to the good-sperm hypothesis, and thus our data are consistent with sexy-sperm, but not good-sperm processes in this species. Sperm viability exhibits additive genetic variance and is heritable in *T. oceanicus* (Simmons and Roberts 2005). Coupled with the significant impact of sperm viability on sperm competitiveness (García-González and Simmons 2005), this provides a mechanism for polyandry to select for increased sperm viability, and therefore competitiveness, in sons of polyandrous mothers. The numerical superiority of viable sperm transferred by a male will, to a large degree, determine his sperm competitiveness. As a consequence, highly sperm competitive sons are expected to be overrepresented within polyandrously sired clutches.

Previous quantitative genetic analyses using *T. oceanicus* have revealed patterns of genotypic variance suggesting a role for maternal effects and/or X-linked inheritance, with little additive genetic variance for offensive sperm competition success (Simmons 2003; Dowling et al. 2010). Such patterns are incompatible with sexually selected sperm processes. There are several possible explanations for these discrepant patterns of additive genetic variation between sperm viability and paternity outcome. First, as mentioned above, fertilization success is not a perfect predictor of sperm competitiveness due to the stochastic and interacting effects of female and competitor male genotypes on paternity outcomes (Clark et al. 1999; García-González 2008). Indeed, sperm viability only explains 35% of the variation in paternity outcome (García-González and Simmons 2005). Thus, the nontransitive nature of sperm competitiveness may reduce observable additive variation in quantitative genetic designs (García-González 2008). Second, individual sperm traits may be negatively genetically correlated (for a review, see Simmons and Moore 2009). For example, in the cockroach, *Nauphoeta cinerea*, negative genetic covariances exist between sperm number and sperm viability (Moore et al. 2004), potentially weakening the additive genetic variance for sperm competitiveness, a polygenic and integrated trait. The extent to which trade-offs between ejaculate traits might affect fertilization success remains unknown in *T. oceanicus*.

The increased sperm viability of sons of polyandrous mothers could also conceivably be explained by genetic incompatibility models (Zeh and Zeh 1996). Polyandrous mothers may be more likely, on average, to fertilize their ova with sperm from a genetically compatible male. Previous work suggests that in some thermal environments, though not others, interactions between sire and dam haplotypes can affect the size of adult male offspring and the size of their testes (Nystrand et al. 2011). However, in a similar study to ours, in which females were mated 3 times, either monandrously or polyandrously, Simmons (2001) found little evidence for a role of genetic incompatibility in driving polyandry in *T. oceanicus*, because polyandrous females did not produce offspring with greater viability, development speed, or realized adult body size. Finally, the increased sperm viability of sons from polyandrous mothers could be due to non-genetic environmental effects. For example, monandrous mothers may potentially receive fewer resources than polyandrous females, if males reduce their reproductive investment with familiar females—the Coolidge effect (Dewsbury 1981). This is unlikely to explain the increased sperm viability of sons of polyandrous mothers found here, as previous research in this (Thomas ML, Simmons LW, unpublished data) and other cricket species (Gershman and Sakaluk 2009) has shown no effect of female familiarity on male reproductive investment. Nonetheless, García-González and Simmons (2007a) showed how paternal effects, most likely mediated via seminal fluid proteins, affect the viability of developing embryos and that these paternal effects can explain the overall increase in viability of embryos produced by polyandrous mothers (Simmons 2001). It is at least feasible therefore, that paternal effects on embryo development have long-term implications for the reproductive quality of adult offspring.

Inconsistent with good-sperm processes, polyandry did not result in increased immunocompetence of offspring, as measured by either immune response or the ability to survive a bacterial infection. Our data did, however, reveal sexual dimorphism in PO activity. PO is a good index of the strength of the invertebrate immune response, given its heritability (Cotter and Wilson 2002) and general correlation with both disease and parasite resistance. However, it is also a multifunctional enzyme that has other roles in insect physiology that should be taken into account when interpreting patterns of sexual dimorphism. Importantly, PO is known to function in egg shell formation and tanning in many invertebrate species (Fetterer and Hill 1994; Bai et al. 1997). Thus, the strong sexual dimorphism in PO observed in sexually mature adults in this study may be significantly affected by a female's investment into egg production, rather than indicative of an inherently elevated immunity.

The relationship between immune function and disease resistance in invertebrates can be complex (for a review, see Adamo 2004b). Why males were better able to survive a live bacterial infection, despite having lower PO and a comparable standing lytic response to females, is not clear and is not consistent with other studies on orthopterans (Adamo et al. 2001). Because immune responses can be pathogen specific (Sadd and Schmid-Hempel 2006), the lytic response, as measured *in vitro* when co-incubated with the gram-positive bacteria *M. luteus*, need not necessarily correlate with an individual's ability to survive an LD_{50} dose of the gram-negative bacteria *S. marcescens*. In the cricket *Gryllus texensis*, the magnitude

of upregulation in lytic activity in response to a non-pathogenic immune challenge, but not the standing lytic levels, predicted a male's ability to survive a subsequent bacterial infection (Adamo 2004a). Whether a similar pattern holds in *T. oceanicus* remains to be tested.

Previous studies that have found support for good-sperm processes report positive correlations between paternal sperm competitiveness and offspring viability using indicators such as juvenile development time (Hosken et al. 2003) and body condition (Simmons and Kotiaho 2002, 2007). We conclude that good-sperm processes can not favor polyandry in *T. oceanicus* because polyandry did not result in increased immunocompetence of offspring. Our conclusion is supported by a previous study of this species which found that highly sperm-competitive fathers did not produce offspring with faster developmental times, adult body sizes, or survival of offspring from hatching until adult emergence (Simmons 2001), 3 other potentially relevant viability indicators.

In conclusion, we show that sons of polyandrous mothers produce sperm of higher viability, a significant predictor of sperm competitiveness. We thereby provide phenotypic support for sexually selected sperm models. The fact that there were no concomitant increases in any measure of offspring immunocompetence is inconsistent with good-sperm models. Ultimately, sexy-sperm models are likely to be inherently difficult to demonstrate because they rely on the absence of correlational relationships, rather than their presence. Yet, by assaying multiple aspects of immunocompetence, an important measure of offspring viability used in precopulatory good-gene models, our study provides exciting evidence for the potential of sexy-sperm processes in this species. In order to show that sexy-sperm processes are involved in the maintenance of polyandry in *T. oceanicus*, we also need to demonstrate a correlation between son's sperm viability and their sister's propensity for multiple mating (Keller and Reeve 1995). Nonetheless, it appears possible that sexy-sperm processes may, in part, drive polyandry in this species.

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