

Age-related sperm transfer and sperm competitive ability in the male hide beetle

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The influence of male age on reproductive success after a single mating has been explored widely; however, few studies have investigated whether quantitative or qualitative differences in male sperm are responsible for the observed patterns. Moreover, the role of male age on sperm competitive ability has been largely ignored. We examined the importance of male age on the probability and amount of sperm transferred during a single mating and explored whether sperm competitive ability varies with male age in the hide beetle *Dermestes maculatus*, a species where sperm viability does not vary with male age. We also investigated whether sperm transfer rates varied with female age. We found that the probability of sperm transfer and the amount of sperm transferred varied with male, but not female, age. All males performed behaviorally successful copulations, but intermediate-age and old males were more likely to transfer sperm successfully and also transferred a greater quantity of sperm than young males. Old males were less likely to transfer sperm than intermediate-age males, but if they did transfer sperm successfully, they transferred comparable amounts. Sperm competitive ability varied with male age and reflected the quantity of sperm transferred. On average, intermediate-age males achieved greater fertilization success when competing against young or old males than when competing against other intermediate-age males. Old males were poor competitors against intermediate-age males, but they achieved significantly higher rates of fertilization when competing against young males. Our findings suggest that quantitative differences in the amount of sperm transferred determine male success in sperm competition in the hide beetle. **Key words:** female age, male age, sperm competition, sperm transfer. [*Behav Ecol* 18:251–258 (2007)]

In theory, female preference for males of particular ages is thought to be maintained largely through the benefits accrued by choosy females (Manning 1985; Hansen and Price 1995; Kokko 1998; Beck and Powell 2000; Proulx et al. 2002). For example, female sand flies *Lutzomyia longipalpis* preferentially mate with intermediate-age males and derive direct fertilization benefits from their choice (Jones et al. 2000). Such fertilization differences may have arisen through quantitative or qualitative variation in male sperm, yet few studies attempt to discriminate between these potential factors, which in insects may include age-related variation in sperm length (Green 2003), sperm quantity (Hayashi 1999), and sperm storage (Taylor et al. 2001). Moreover, by focusing on the consequences of female mating preferences, the relative importance of female age and the opportunity for sperm competition to influence the outcome of reproductive events has been largely ignored.

Most studies that explore age-related variation in male mating success use a single mating attempt to determine the reproductive potential of males in specific age classes (e.g., Delisle 1995; Fox et al. 1995; Rogers and Marti 1997; Jones et al. 2000; Jones and Elgar 2004; Wedell and Ritchie 2004). Although this may be appropriate for monandrous species, it reveals little about the reproductive success of males of different age classes in polyandrous species. This may be particularly pertinent if polyandry biases paternity toward the most competitive and highest quality males (Tregenza and Wedell 2002). Studies that have explored variation in sperm competitive ability report a range of outcomes. The likelihood of

being cuckolded decreases with male age in the brown thornbill *Acanthiza pusilla* (Green et al. 2002), and second male sperm precedence is more prevalent in the noctuid moth *Heliothis virescens* if the second male to mate is older than the first (LaMunyon 2001). In contrast, old male cellar spiders *Pholcus phalangoides* have reduced fertilization success if they are the second to mate (Schafer and Uhl 2002), and young male bulb mites *Rhizoglyphus robini* outcompete their older rivals in sperm competition trials (Radwan et al. 2005). Selection experiments have also revealed evidence of senescent decline in sperm competitive ability in the fruit fly *Drosophila melanogaster* (Service and Fales 1993). However, these studies cannot determine whether the variation in success in sperm competition arises through qualitative or quantitative differences in the sperm transferred. In the only study to hold sperm quantity constant, Hoysak et al. (2004) found no evidence that sperm competitive ability of sockeye salmon *Oncorhynchus nerka* varied with male age or mating position. Nevertheless, it is unclear whether fertilization success in this species actually varies with male age (Hoysak and Liley 2001).

Female age may also influence the probability of copulation or subsequent patterns of fertilization. Female mating success in the oblique-banded leaf roller *Choristoneura rosaceana* declines with age (Delisle 1995). In contrast, the number of cloacal contacts and male mounting rates in the common tern *Sterna hirundo* were positively associated with female age (Gonzalez-Solis and Becker 2002). These studies do not investigate explicitly the effect of female age on fertilization success; however, female cockroaches *Nauphoeta cinerea* that were older when first mated laid fewer offspring per clutch and fewer clutches than young mated females (Moore et al. 2001). Finally, last-male precedence declines with increasing female age in the fruit fly *D. melanogaster* (Mack et al. 2003). To date, no study has provided an adequate mechanism to explain these patterns, although a wealth of possibilities have

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been proposed (for comprehensive reviews, see Birkhead and Møller 1998; Simmons 2001).

Here, we assess the importance of female and male age on the quantity of sperm transferred to a female and explore whether sperm competitive ability varies with male age in the hide beetle *Dermestes maculatus* De Geer (Coleoptera: Dermestidae). The hide beetle is a small, carrion-feeding insect that forms aggregations on spatially and temporally patchy resources where individuals feed and mate (Archer and Elgar 1998). The influence of female age on male reproductive behavior is unknown, but male reproductive success varies with age. Seven-week-old males accrue more matings than 13-week- or 11-day-old males when in small competitive mating aggregations (Jones and Elgar 2004). Moreover, single-mated females accrue direct fertilization benefits—they lay larger clutches and fertilize a greater number and proportion of eggs—when mating with 7-week-old males (Jones and Elgar 2004). Recent data indicate that sperm viability, a qualitative measure of sperm, does not vary with male age (Hale J, unpublished data); however, although not tested explicitly, quantitative differences are thought to explain the observed low fertilization success of the youngest and oldest males (Jones and Elgar 2004). The effect of male age on sperm competitive ability has never been studied.

The aim of this study was twofold. First, we explored whether the quantity of sperm transferred by a male to a female during mating varied with male or female age. Second, we assessed the sperm competitive ability of males varying in age, by assessing the fertilization success of each of 2 males who were either the same or different age when mating with a single female of known age.

METHODS

Culturing

Adult beetles were obtained from a large laboratory population that had been maintained for 15 generations at the University of Melbourne. Individuals were kept in a temperature-controlled room at 28 °C, on a 12:12 h light:dark cycle and fed *ad libitum* on a diet of dried bonemeal and water. Pupae were transferred to individual 5-ml vials prior to adult emergence to ensure virginity. On emergence, experimental adults were housed individually in plastic rearing containers (diameter = 68 mm, height = 28 mm) containing dried bonemeal and water and were maintained at 25 °C on a light and food regimen identical to the immature stages of the life cycle. Experimental animals were standardized for size (mean \pm standard error [SE] female weight = 0.031 ± 0.002 g, female length = 5.29 ± 0.03 mm, male weight = 0.029 ± 0.001 g, male length = 5.21 ± 0.02 mm) but varied in age (see treatment groups below).

Age classes

On eclosion, males and females were assigned to 1 of 3 age categories (young, intermediate age, or old) and then maintained with *ad libitum* food and water in individual rearing containers (as above) for 9.78 ± 0.05 days ($N = 63$ young males), 9.50 ± 0.37 days ($N = 23$ young females), 41.96 ± 0.10 days ($N = 70$ intermediate-age males), 42.34 ± 0.11 days ($N = 123$ intermediate-age females), 91.78 ± 0.12 days ($N = 65$ old males), or 91.30 ± 0.19 days ($N = 22$ old females). These 3 age classes incorporate a large component of the life span of this species under laboratory conditions, as male hide beetles survive and will continue to mate for more than 4 months in the laboratory (Archer and Elgar 1998). All males were maintained as virgins until used in the experiment to ensure identical mating histories; however, a previous study

found no evidence that fertilization success varied with male mating history, and thus, male age and mating experience are unlikely to be confounded (Jones and Elgar 2004).

The effects of age on sperm transfer

To explore factors influencing sperm transfer, 69 females of known age class were assigned a male of known age class. Pairs were introduced to a petri dish (30-mm diameter, 10-mm height) and left to copulate. After a successful mating, the female was returned to her individual container and left for 40 min before being decapitated. The female reproductive tract was dissected out in 0.9% phosphate-buffered saline (PBS) solution (Sigma, Castle Hill, New South Wales, Australia). The spermatheca was transferred to a standard glass slide with 0.2 ml of 0.9% PBS and teased apart with fine dissecting needles to release the stored sperm. The slide was covered with a 22×22 -mm glass coverslip, examined twice under a light microscope ($\times 100$ magnification), and scored for the presence and quantity of sperm. In the hide beetle, sperm are transferred in bundles, each containing 256 spermatozoa (Al-Taweel and Fox 1983). Separation of the sperm bundles using conventional techniques (Yusa 1996; Hayashi 1998; Hellriegel and Bernasconi 2000) was unsuccessful, and so we applied a rank measure of the amount of sperm transferred. Rank 0 = no sperm transferred, rank 1 = sperm was detected on the slide but no distinct sperm bundles were transferred, rank 2 = distinct sperm bundles were transferred but these covered less than 50% of the coverslip, and rank 3 = distinct sperm bundles were transferred that covered more than 50% of the coverslip. The distinction between ranks 2 and 3 was always unambiguous; the amount of sperm transferred was either substantially less than 50% coverage or close to 100%. Where sperm was not found, the male reproductive organs were dissected out within 24 h after mating; the male's mature sperm storage organ (the seminal vesicle) was removed and separated with dissecting needles on a glass slide and examined for the presence of sperm. Males that transferred sperm were discarded without dissection.

The effects of male age on fecundity and sperm competition

To investigate the relationships between sperm competitive ability, male age, and mating behavior, 2 males of similar or different ages were mated to a single virgin female and the outcome of sperm competition was assessed. A total of 98 intermediate-age virgin females were assigned to 1 of 9 female mating combinations consisting of 2 males of either similar or different ages. Each male was permitted a single copulation only, and males of all 3 age classes competed for fertilization success with males from every other age class (see Figure 3 for samples sizes). For each mating, an individual female was introduced to a petri dish (30-mm diameter, 10-mm height) with their first male. After a successful copulation, this male was removed and the process repeated with a second male.

Standard sterile male techniques were used to infer patterns of paternity (Boorman and Parker 1976). This technique has been used successfully with no evidence of an irradiation effect on fertilization success (Archer and Elgar 1999). Twenty-four hours prior to the commencement of a trial, 1 of the 2 males within a pair was subjected to a sublethal dose of radiation (3.5 krad from a Cobalt-60 source) that renders sperm capable of fertilization but prevents embryo development. A balanced reciprocal design was used to control for the potential effects of irradiation, mating order, and age.

To assess fecundity and paternity success, mated females were provided with a water-soaked sponge (1×1 cm), a piece of ox liver (0.5×0.5 cm), and a piece of synthetic fur on which to lay eggs (3×3 cm). Food and water were replenished every 3 days; fur was replenished every second day for a total of

6 days. As female hide beetles can lay fertilized eggs for several weeks after a mating (Archer and Elgar 1999), this time period was short enough that sperm depletion would have no impact on the observed patterns of paternity. At 25 °C, egg viability can be determined by embryo pigmentation 48 h after oviposition (after Archer and Elgar 1999). For all females, eggs that developed characteristic eyespots were assigned to the nonirradiated male; eggs that remained opaque were assigned to the irradiated male. Females that failed to produce eggs within 6 days were dropped from analyses of fecundity and fertilization success as the majority (23 of 24 females) of female hide beetles commence oviposition within 30 h after their first mating (Featherston R, unpublished data).

Statistics

Variation in the probability of initiation of copulation, rank sperm transfer, and the number of eggs sired was analyzed using analyses of covariance (ANCOVAs) in JMP 4.0.2 (SAS Institute Inc, Cary, NC); data were transformed where necessary to achieve normality. Analyses exploring variation in the proportion of eggs sired were performed in MlwiN 1.2 (Rasbash et al. 2000) using a binomial response model with a logit-link function. This model included the total number of eggs laid as a denominator, thus accommodating the large observed variation in female fecundity. Significance levels were determined using Wald (χ^2) tests.

We avoided incorporating pseudoreplication in our analyses of the variation in fecundity and fertilization success in sperm competition trials, by using the fertilization success (number and proportion of eggs sired) of the second male only from any mating pair. The results were qualitatively identical if the analyses were performed using only the first male to mate. Male irradiated status was included initially in all models but was dropped because it did not significantly explain any variation. We carried out planned comparisons, after our ANCOVA or binomial models, because we were interested only in how a particular male age class performed against other age classes (e.g., young males vs. young, intermediate-age, or old males: Quinn and Keough 2002). Unless otherwise stated, all presented averages are means \pm SEs.

RESULTS

The effects of male and female age on sperm transfer

Presence of sperm

The probability of a male having sperm present in his mature sperm organ varied across the 3 age classes ($\chi^2_2 = 7.61$, $P = 0.02$). All intermediate-age ($n = 23$) and old ($n = 24$) males had mature sperm present in their seminal vesicles; in contrast, mature sperm was found in the seminal vesicles of only 36% of young males ($n = 22$).

Copulation duration

All males performed behaviorally successful copulations (mean duration of copulation \pm SE = 58.31 \pm 4.05 s). There was no relationship between copulation duration and either male or female age (effect of male age: $F_{2,58} = 0.77$, $P = 0.47$; female age: $F_{2,58} = 0.01$, $P = 0.99$) and no significant interaction between male and female age classes ($F_{4,58} = 2.28$, $P = 0.07$). Copulation duration was also unrelated to either male or female weight (effect of male weight: $F_{1,58} = 0.007$, $P = 0.79$; female weight: $F_{1,58} = 0.003$, $P = 0.96$).

Probability of sperm transfer

Although copulations were behaviorally successful, not all males transferred sperm during copulation. The probability

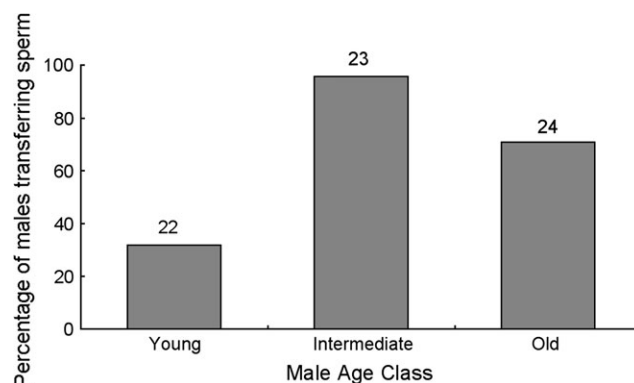


Figure 1

The percentage of young, intermediate-age, and old males transferring sperm to young, intermediate-age, and old females during a single mating. Sample sizes are shown above the individual bars.

of a male transferring sperm to a female's spermatheca varied with his age (nominal logistic model: $\chi^2_2 = 14.70$, $P = 0.0006$; Figure 1): intermediate-age and old age males were more likely to transfer sperm than young males (post hoc tests, both $P < 0.0001$) and intermediate-age males were also more likely to transfer sperm than old males ($P = 0.04$). All females were equally likely to receive sperm irrespective of their age ($\chi^2_2 = 1.57$, $P = 0.46$). There was no relationship between the probability of sperm transfer and copulation duration ($\chi^2_1 = 0.06$, $P = 0.81$), male weight ($\chi^2_1 = 1.14$, $P = 0.28$), or female weight ($\chi^2_1 = 1.00$, $P = 0.32$).

Rank amount of sperm transferred

If all males are considered, the rank amount of sperm received by a female after a behaviorally successful mating depended on the age of her mate ($F_{2,57} = 27.83$, $P < 0.0001$; Figure 2a). Intermediate-age males transferred more sperm than either older or younger males (post hoc Student's *t*-tests, both $P < 0.05$); old males also transferred more sperm than young males (post hoc Student's *t*-test, $P < 0.05$). Females received comparable amounts of sperm irrespective of their age ($F_{1,57} = 0.53$, $P = 0.59$). There was no interaction between male and female age with respect to the amount of sperm transferred ($F_{4,57} = 1.33$, $P = 0.27$). There was also no significant effect of male weight ($F_{1,57} = 1.90$, $P = 0.17$), female weight ($F_{1,57} = 0.28$, $P = 0.60$), or copulation duration ($\chi^2_1 = 0.33$, $P = 0.57$) on the rank amount of sperm transferred.

If only those males that transferred sperm successfully are considered, the amount of sperm transferred by a male varied with his age ($F_{2,38} = 9.20$, $P = 0.0006$; Figure 2b). Post hoc Student's *t*-tests revealed that intermediate-age and old males transferred more sperm than young males (both $P < 0.05$); but intermediate-age and old males transferred comparable rank amounts of sperm ($P > 0.05$). There was a trend suggesting that the amount of sperm transferred varied with female age ($F_{2,38} = 2.70$, $P = 0.08$), but there was no significant effect of male weight ($F_{2,38} = 0.08$, $P = 0.78$), female weight ($F_{2,38} = 1.08$, $P = 0.31$), or copulation duration ($F_{2,38} = 0.59$, $P = 0.45$). In this analysis, it was not possible to include the interaction between male age and female age because the 7 young males that transferred sperm did so to young and old females only.

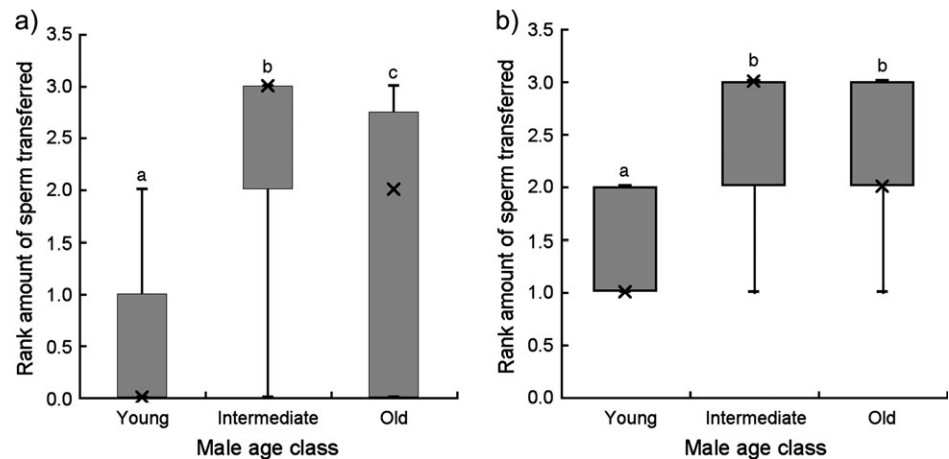
The effects of male age on fecundity and sperm competition

Copulation duration

There was no difference in the duration of copulation between the first and second male to mate with a female (mean

Figure 2

The average rank amount of sperm transferred to a female after a single mating with a young, intermediate-age, or old male for (a) all males ($n = 22$ young males, 23 intermediate-age males, and 24 old males) and (b) only males that transferred sperm ($n = 7$ young males, 22 intermediate-age males, and 17 old males). The box plots denote the interquartile range about the median (\times); the whiskers denote the maximum and minimum values. Significant differences between treatment groups are denoted by different letters.



duration of first copulation = 51.41 ± 2.84 s, second copulation = 47.39 ± 2.69 s, matched pairs t -test: $t = 1.03$, $N = 99$ females, $P = 0.31$). This pattern was consistent across the 9 male mating groups ($F_{8,90} = 1.23$, $P = 0.29$).

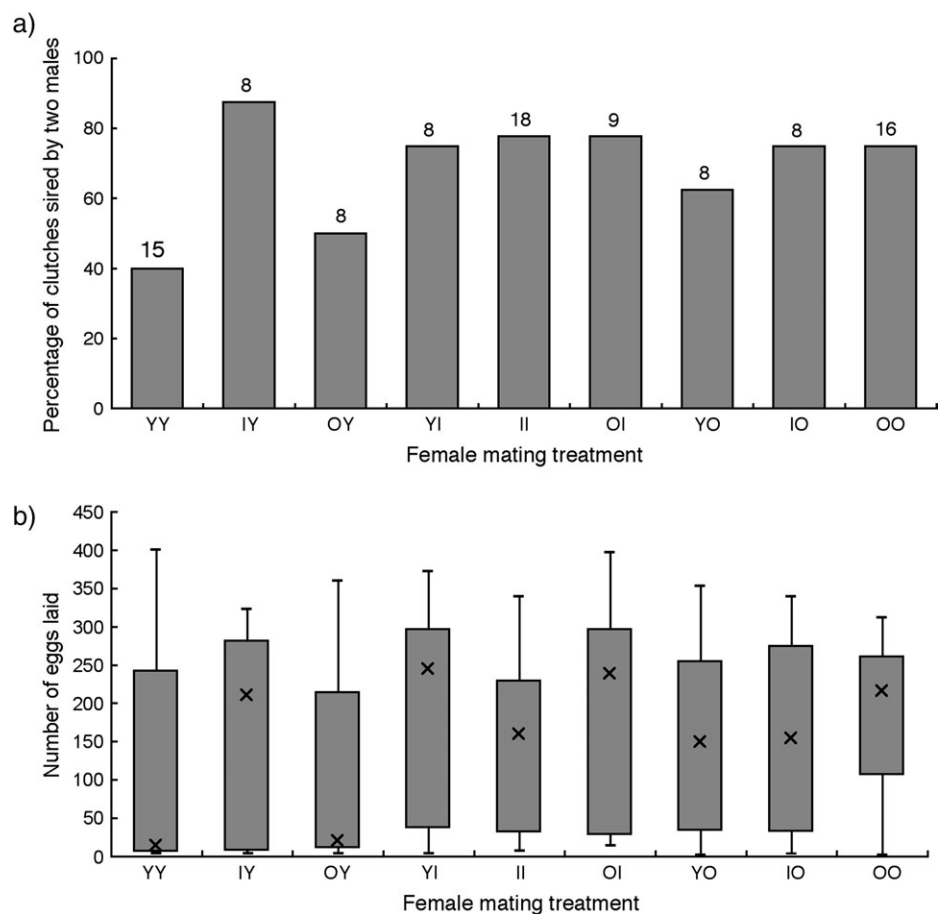
Fecundity

Three twice-mated females (1 IY female and 2 IO females) that failed to commence oviposition after 6 days were dropped from subsequent analyses.

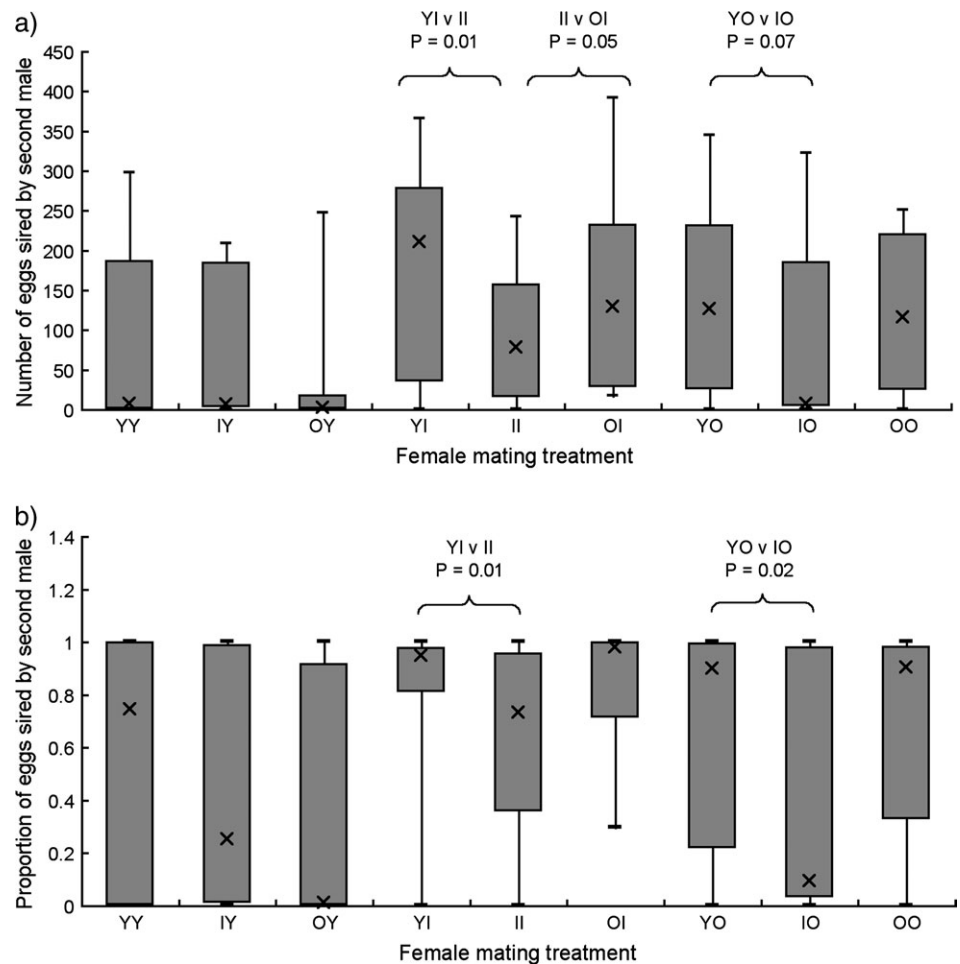
There was no overall difference between the groups in the percentage of clutches with 2 sires (nominal logistic regres-

sion: $\chi^2_8 = 9.18$, $P = 0.33$; Figure 3a). However, notably, only 40% of the females that mated to 2 young males (YY females) produced clutches with 2 sires.

The mean number of eggs laid was comparable across the 9 female mating treatments ($F_{8,76} = 0.34$, $P = 0.95$; Figure 3b), but clutches sired by 2 males were considerably larger than clutches sired by a single male (mean clutch size of females with a single sire = 23.29 ± 5.62 , $N = 31$ females; 2 sires = 216.8 ± 12.6 , $N = 67$ females; $F_{1,76} = 73.48$, $P < 0.0001$). Neither the interaction between female mating treatment and the number of sires per clutch ($F_{8,76} = 0.29$, $P = 0.97$)

**Figure 3**

The (a) percentage of twice-mated females producing egg clutches sired by 2 males and (b) number of eggs laid by females from each of the 9 female mating treatments. Notation is as follows: Y = young males, I = intermediate-age males, and O = old males. The order of letters in the female mating treatment corresponds to the order in which a male mated. Sample sizes are shown above the individual bars in (a) and are identical for (b). The box plots in (b) denote the interquartile range about the median (\times); the whiskers denote the maximum and minimum values.

**Figure 5**

The (a) mean number of eggs sired and (b) proportion of eggs sired by second mating males from each of the 9 female mating treatments. The box plots denote the interquartile range about the median (×); the whiskers denote the maximum and minimum values. Notation and sample sizes as in Figure 3. Significant differences after planned comparisons within an age class are shown above the bars.

lower than for species with a monandrous mating strategy (Jones 2001).

Our study confirms that, in the hide beetle, age-related variation in fertilization success is correlated with sexual maturation and is determined, at least in part, by numerical representation of sperm in a female's reproductive tract (*sensu*, the raffle model, Parker 1990) and failure of the males to transfer sperm (*sensu*, Garcia-Gonzalez 2004). Intermediate-age males transferred consistently the greatest amount of sperm in a single mating and were highly successful in sperm competitive interactions against young and old males. Conversely, young males transferred small amounts or no sperm and fared poorly in sperm competition trials. Moreover, old males were less likely to transfer sperm during mating and have lower fertilization success than intermediate-age males, even in the absence of competition (Jones and Elgar 2004), although it is worth noting our rank measure of quantity was conservative, particularly at the upper limit of our rank. We did not test explicitly whether the quality of sperm produced by the oldest males was also reduced; however, preliminary evidence suggests that sperm viability, a recently employed measure of sperm quality (Garcia-Gonzalez and Simmons 2005; Harris and Moore 2005), does not vary with male age (Hale J, unpublished data). Whether other measures such as sperm motility or survival (Kidd et al. 2001) are more relevant indicators of sperm quality in the hide beetle remains to be investigated. We similarly cannot confirm that single-sired clutches reflect a failure to transfer sperm *per se* rather than the failure of transferred sperm to fertilize a female's eggs.

However, this appears conceivable given that the percentage of double-male clutches within each female mating treatment correlated closely with the overall probability that the 2 males in that group would transfer sperm (Figure 4).

Age-specific variation in male fertilization success has several implications for females. Given that young and old males provide females with inadequate sperm for fertilization (see Jones and Elgar 2004), selection should favor females that are able to discriminate against these age classes. This may be the case in the hide beetle: in competitive mating aggregations, young and old males lose mating opportunities to intermediate-age rivals (Jones and Elgar 2004), although this pattern may be difficult to interpret in species in which it is difficult to distinguish between male performance and female preference (Jones et al. 2006). However, female hide beetles are highly polyandrous (Archer and Elgar 1999) and gain direct fertilization benefits from a remating or multiple-mating strategy (McNamara KB, unpublished data). This polyandrous mating strategy may allow females to avoid fertilization by older males with potentially lower quality sperm as intermediate-age males are more likely to outcompete their older and young rivals in sperm competition (see also, Radwan 2003; Radwan et al. 2005). Whether female hide beetles have any influence on patterns of sperm utilization after mating (Eberhard 1996) or whether the observed weak effect of female age on the amount of sperm transferred has any subsequent effect on the outcomes of sperm competition (Eberhard 1996; Mack et al. 2003) require further investigation.

Finally, we cannot exclude the possibility that the quality and/or quantity of seminal products, transferred during

mating, also covaries with male age. Reports from 59 insect species, covering 6 orders, suggest that seminal products may influence female reproduction and oviposition behavior (Eberhard 1996). Moreover, deterioration of accessory products responsible for agglutinating individual sperm into bundles could conceivably impair successful sperm transfer or affect sperm storage (Simmons 2001). This may impede sperm movement through a female's viscous reproductive tract (Trivers 1985; Hayashi 1998) and have particularly severe consequences for males if the likelihood of sperm competition is high (Scott and Richmod 1990; Simmons 2001).

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