The role of male age, sperm age and mating history on fecundity and fertilization success in the hide beetle

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Models of age-related mate choice predict female preference for older males as they have proven survival ability. However, these models rarely address differences in sperm age and male mating history when evaluating the potential benefits to females from older partners. We used a novel experimental design to assess simultaneously the relative importance of these three parameters in the hide beetle, *Dermestes maculatus*. In a two-part experiment we first explored age-related male mating success and subsequently examined the consequences of male age, sperm age and male mating history on female fecundity and fertilization success. In a competitive mating environment, intermediate-age males gained significantly higher mating success than younger or older males. To test the consequences for females of age-related male mating success, a second set of females were mated to males varying in age (young, intermediate-age and old), in numbers of matings and in timing of the most recent mating. We found that male age had a significant impact on female fecundity and fertilization success. Females mated to intermediate-age males laid more eggs and attained consistently higher levels of fertilization success than females with young and old mates. A male's previous mating history determined his current reproductive effort; virgin males spent longer *in copula* than males with prior mating opportunities. However, differences in copulation duration did not translate into increased fecundity or fertilization success. There was also little evidence to suggest that fertilization success was dependent on the age of a male's sperm. The experiment highlights the potential direct benefits accrued by females through mating with particular aged males. Such benefits are largely ignored by traditional viability models of age-related male mating success.

Keywords: male age; sperm age; mating history; fertilization success

1. INTRODUCTION

Viability indicator models of age-related mate choice predict that females should prefer older males as mates because they have proven survival ability or because their signals are potentially more revealing (Kokko & Lindstrom 1996; Kokko 1997; Proulx *et al.* 2002). Such models argue that females use age as a reliable signal of male quality and that, by mating with older males, they gain indirect benefits through the production of higher-quality offspring. While viability indicator models have gained empirical support (see Brooks & Kemp (2001) for a recent review), direct trade-offs between fitness components and age-specific differences in survival may reduce the fertility of older males and instead promote the evolution of female preference for young and intermediate-age males (Hansen & Price 1995, 1999; Kokko 1997; Beck & Powell 2000; Beck *et al.* 2002).

Female discrimination against older males has been reported in a few species (Burley & Moran 1979; Ritchie *et al.* 1995; Jones *et al.* 2000). Maintenance of female preference for younger and intermediate-age males would be further strengthened if the reproductive fitness of females mating with older individuals declined owing to age-related reductions in sperm quality through, for example, mutagenesis (Crow 1997; Hansen & Price 1999). Age-related declines in mating and fertilization success have

been reported for a wide range of species (Clutton-Brock 1988; Price & Hansen 1998; Jones *et al.* 2000; Kidd *et al.* 2001; Bonduriansky & Brassil 2002). Moreover, females mating with intermediate-age and younger males may gain direct benefits through increased fertility (Jones *et al.* 2000) and/or indirect benefits via improved offspring viability (Price & Hansen 1998). However, to our knowledge, no study has established convincingly what mechanisms are responsible for these observed reductions in fertilization success or offspring viability.

A potentially confounding issue that has received remarkably little attention is that studies investigating age-related fertilization success rarely standardize male mating history. As a consequence, variation in the quantity and quality of sperm may confound the specific effects of male age (Siva-Jothy 2000). If sperm quality declines with age through damage to the DNA or the spermatozoan cell membrane (Vishwanath & Shannon 1997; Ahmadi & Ng 1999; Irvine *et al.* 2000), females mating with males who have older stored sperm may suffer reduced fertility. Studies reporting age-specific declines in fertilization success or offspring viability using virgin males (for example Price & Hansen 1998; Jones *et al.* 2000) may thus be observing the negative effects of spermatozoan age on zygote viability rather than the effects of male age on spermatogenesis (Crow 1997). Alternatively, sperm age may be independent of male age; successful males may replenish their sperm frequently and so have better fertilization success than less successful competitors, regardless of age (Siva-Jothy 2000). A further complexity is that females

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mating with highly successful males may suffer lower reproductive success because of the effects of sperm depletion (Jones 2001; Preston *et al.* 2001; Wedell *et al.* 2002). This may be further confounded if sperm quantity varies with periods of abstinence from mating events irrespective of sperm quality (Mortimer *et al.* 1982; Pellestor *et al.* 1994; Noirault & Brillard 1999). Ultimately, females may be required to trade-off male age against sperm age and mating history when choosing a mate. Surprisingly, to our knowledge, no study has attempted to explore the effects of these factors independently.

Here, we assess the importance of male age, sperm age and male mating history in determining fertilization success 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 & Elgar 1998). Natural aggregations vary in size depending on the food source, but on a small carrion source they typically range from 1 to 13 adults (mean $\pm$ s.e. = 2.37 ± 0.5 , $n = 6$ carcasses; M. Archer, personal communication). Male hide beetles possess a pheromone gland on the base of their abdomen that elicits an aggregation response in both sexes (Abdel-Kader & Barak 1979; Rakowski & Cymborowski 1986). In the laboratory, both males and females mate multiply and females commence oviposition within 24 h following their first mating ($n = 27$ females; R. Featherston, personal communication). Females are capable of ovipositing continuously throughout their four-to-six-month lifespan and will, given the opportunity, remate (Archer & Elgar 1999). Although males frequently initiate matings (K. B. McNamara, unpublished data), females exhibit some degree of choice and can reject unwanted copulation attempts by either walking away or throwing a male off her back (Archer & Elgar 1999).

In a two-stage experiment, we first assessed age-related male mating success in a series of mating trials with males varying in age. Then, to disentangle the relative impact of male age, sperm age and male mating history on fertilization success, we compared measures of behaviour, fecundity and fertilization success across nine groups of females mated to males of varying ages and mating histories. The experimental design allowed us to make several predictions with respect to male age, sperm age and male mating history. Consistent changes in age-related reproductive success irrespective of mating history or sperm age indicate that male age is an important factor promoting mating success in this species. Differences in the reproductive success of females with respect to the mating history of her mate provides an indication of the importance of recent and past reproductive events on a male's current fertility. Finally, interactions between male age, sperm age and male mating history may shed light on the possible mechanisms promoting the observed age-related declines in fertilization success found in previous studies (e.g. Price & Hansen 1998; Jones *et al.* 2000).

2. METHODS

(a) *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 controlled-temperature room at 28 °C, on a 12 L : 12 D cycle and fed *ad libitum* on a diet of dried meat meal 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 (68 mm diameter, 28 mm high) containing bone-meal and water and were maintained at 25 °C on an identical light and food regime to the immature stages of the life cycle. Experimental females were standardized for age and size (mean age $\pm$ s.e. = 39.4 ± 0.38 days; mean body length $\pm$ s.e. = 7.44 ± 0.04 mm). Males were standardized for size (mean body length $\pm$ s.e. = 7.03 ± 0.04 mm), but varied in age (see treatment groups below).

(b) *Male age and mating regimes*

Upon eclosion, males were assigned to one of three age categories (young, intermediate-age or old) and then maintained with food and water *ad libitum* in individual rearing containers (as above) for 11.5 ± 0.26 days (young males), 47.4 ± 0.37 days (intermediate-age males) or 88.3 ± 0.71 days (old males). These three age classes incorporate a large component of the lifespan of this species under laboratory conditions, as male hide beetles survive and will continue to mate for over four months in the laboratory (Archer & Elgar 1998). Within each age category, males were allocated to one of three mating regimes (virgin, 'V', single mating, 'SM', or lifetime mating, 'LM'). V males were maintained in isolation without mating until they reached their experimental age (young, intermediate-age or old); thus sperm was likely to be retained and aged for increasing periods of time, dependent on the age of the male. SM males were maintained in an identical manner to V males up to 48 h prior to testing. At this time they were provided with three virgin females. Females were removed 24 h later and the males provided with a 24 h 'recovery period' prior to their test mating. Under this regime, SM males were expected to produce new sperm for subsequent matings. In the LM male group, males were provided with three females per week throughout their reproductive lives. Females were removed 24 h prior to a male's test mating to permit a recovery period. Thus, within the V and SM categories, male mating history was standardized. However, sperm age was expected to correlate with age for V males but be similar across all age classes for SM males. By contrast, in the LM category, mating history varied with respect to male age, but sperm age was expected to be similar across all males. Actual copulations were not recorded during this pre-experimental period; however, following removal from the male, females were placed into fresh containers and permitted to oviposit. The presence of offspring in the majority (89%) of female containers confirmed that males had mated with at least one of the three females.

(c) *Experimental protocol*

(i) *Age-related male mating preference*

To assess age-related male mating success, we established 10 sets of three-male aggregations consisting of one young, one intermediate-age and one old male. SM males were used in the experiment to ensure that although males varied in age they had identical recent mating histories. Within each aggregation, males were individually marked, placed in a Petri dish (85 mm diameter, 12 mm high) and permitted a 10 min acclimatization period. A single virgin female was subsequently introduced into the centre of the arena and left for 30 min or until she had mated. After this time the female was removed from the arena and replaced after a rest period of 30 min with another virgin

Table 1. The number of males contributing to behavioural, fecundity and fertilization analyses across the nine male-mating regime and male-age categories.

(Males were mated to a single intermediate-age, virgin female and then discarded following mating. Mated females were subsequently permitted to oviposit for 6 days.)

mating regime	age class	males (<i>n</i>)	copulations (<i>n</i>)	females ovipositing (<i>n</i>)
V males	young	32	28	11
	intermediate-age	38	32	16
	old	28	22	11
SM males	young	30	28	15
	intermediate-age	41	34	20
	old	21	20	13
LM males	young	35	30	18
	intermediate-age	36	28	19
	old	23	20	11

female. This was repeated for a total of six females per aggregation of three males. Approach and rejection behaviours were noted for males and females regardless of whether copulation occurred. For each mating event, the identity of the mating male and the length of copulation were recorded.

Behavioural data were analysed in JMP v. 4.0 (SAS Institute 1989–2000) using nested ANOVAs. Male age and aggregation were added as categorical variables with 3 and 10 levels, respectively. Female order was added as an additional variable nested within trial. The proportion of matings obtained by each male in his aggregation was compared using a multi-level approach in MIWin v. 1.2 (Multilevel Models Project, Institute of Education 2002). A two-level model was constructed with female and aggregation as the first and second levels, respectively. As the number of matings per aggregation varied, a binomial error distribution was assumed, which includes the total number of matings per aggregation as a denominator in the model. Male age was included as a categorical variable with three levels. Significance levels were determined with Wald tests. Presented means and standard errors were calculated from the predicted estimates generated by the model.

(ii) Fertilization trials

To explore simultaneously the effect of male age and mating history on reproductive success, a total of 284 females was randomly assigned to a single male from one of the nine treatment groups (see table 1 for sample sizes). Females were introduced individually with a male to a small plastic vial (35 mm diameter, 50 mm high) and permitted to mate. The time to mating and the duration of mating were recorded. Following mating, females were placed in a fresh plastic rearing container, supplied with a piece of synthetic fur (20 mm²), fresh pigs' liver (10 mm³) and a water-soaked piece of sponge (10 mm³), and permitted to oviposit for 6 days. During this period the fur was changed every 2 days to minimize cannibalism by earlier hatched larvae (Archer & Elgar 1999). The numbers of eggs laid and fertilized by each female were recorded. The proportion of fertile eggs, determined by the presence of an eye-spot 3 days after oviposition (Archer & Elgar 1999), was calculated from these data.

Data (excluding the proportion of eggs hatching) were analysed using standard least-squares or logistic regression models provided by the model fitting platform in JMP v. 4.0 (SAS Institute) and were log-transformed where necessary to achieve normality. Females that did not commence oviposition were discarded from analyses of fecundity and fertilization success. Male age, mating regime and the interaction between male age and

mating regime were included as variables in all models. Male and female body size were included as parameters in copulation models; female size was also incorporated into models of fecundity but was dropped from models examining fertilization success as it was correlated with the number of eggs laid. The duration of copulation was also incorporated into models of fecundity and fertilization success. The total number of eggs laid was included as an additional parameter in the model of mean number of eggs hatched. The proportion of eggs hatching was compared using a single-level model (with individual female as the first level), in MIWin v. 1.2 (Multilevel Models Project, Institute of Education 2002). As the number of eggs laid per female varied, a binomial error distribution was assumed that included the total number of eggs laid as a denominator in the model. Male age, mating regime, the interaction between male age and mating regime, and copulation length were included as variables in the model. Presented means and standard errors are calculated from the predicted estimates generated by the models. Significance levels were determined by using Wald tests. Differences in sample size in some models are a result of missing values for male and female body size.

3. RESULTS

(a) Female preference trials

On average, only 32.1% (s.e. $\pm 0.07\%$) of females introduced into mating arenas copulated within 30 min. Successful copulations were more likely to be initiated by males than by females (number of matings initiated by males = 18, by females = 6; $\chi^2_1 = 6.0$, $p = 0.01$). However, males of all ages were equally likely to approach or reject females (Wilcoxon test on the number of male approaches to females in each of the three male-age classes: $\chi^2_2 = 1.32$; the number of rejections by males from each age class: $\chi^2_2 = 0.44$; both $p > 0.30$). Moreover, females were equally likely to approach and reject all males regardless of age class (Wilcoxon test on the number of approaches by females to males in each age class: $\chi^2_2 = 0.44$; the number of times that females reject males from each age class: $\chi^2_2 = 4.09$; all $p > 0.15$).

The full multi-level model revealed that, within the mating aggregations, intermediate-age males had a higher mating success than the young or old males (table 2; figure 1). However, the proportion of matings obtained by a male was independent of the number of times he was approached or rejected by the six females in his trial

Table 2. Age and behavioural traits explaining variation in the number of matings obtained by the three males in three-male mating aggregations consisting of one young, one intermediate-age and one old male.

(Data were analysed using a multi-level approach in MIWin v. 1.2 (Multilevel Models Project, Institute of Education 2002). Significance levels were determined with Wald tests. Means and standard errors were calculated from predicted estimates generated by the model.)

parameter	mean $\pm$ s.e.	χ^2	<i>p</i> -value
male age class	see figure 1	8.77	0.01
number of female approaches	4.2 $\pm$ 0.33	0.41	0.52
number of male approaches	1.73 $\pm$ 0.37	1.18	0.27
number of female rejections	0.57 $\pm$ 0.14	1.16	0.28
number of male rejections	0.73 $\pm$ 0.21	0.01	0.92

Figure 1. The mean proportion of matings obtained by young, intermediate-age and old males when competing in three-male aggregations ($n = 13$ trials). Each three-male aggregation comprised one young, one intermediate-age and one old male. A total of six intermediate-age females were introduced sequentially into each aggregation and permitted to mate. Error bars represent standard errors about the mean. Means and standard errors were obtained from predicted values taken from a logistic model of male mating success in MIWin v. 1.2 (Multilevel Models Project, Institute of Education 2002). Significance levels between the male age groups are denoted as $*p < 0.05$ and $**p < 0.01$.

(table 2). Moreover, a male was equally likely to approach and reject a female regardless of whether he mated with her (table 2). All but one of the observed 'rejection' behaviours involved individuals walking away from an approaching opposite-sex conspecific. On a single occasion, a young male attempted to remate with the same female immediately after his first copulation, but the female rejected him immediately by raising her abdomen and throwing him off her back.

The time taken prior to initiation of a successful copulation varied with male age (mean $\pm$ s.e. time to copulation for young males = 212.4 $\pm$ 68.7, intermediate-age males = 282.8 $\pm$ 50.4, old males = 68.2 $\pm$ 81.3; $F_{2,24} = 6.84$, $p = 0.03$). *Post hoc* Student's *t*-tests revealed that older males commenced copulating more rapidly than intermediate-age males ($p < 0.01$). All other pairwise comparisons were non-significant (both $p > 0.05$). The duration of copulation was consistent across the three male age classes (mean $\pm$ s.e. duration of copulation for young males = 65.3 $\pm$ 14.0, intermediate-age males = 77.4,

old males = 77.0 $\pm$ 20.0; $F_{2,24} = 0.26$, $p = 0.61$). Both behaviours measured were independent of female mating order (both $p > 0.20$) and were consistent across the 10 aggregations (both $p > 0.30$).

(b) Fertilization trials

(i) Copulation

A total of 15.1% males failed to mate with a female within 30 min, but there was no effect of either mating regime or male age on the probability of mating (table 1; mating regime: $\chi^2_2 = 1.74$; male age: $\chi^2_2 = 2.22$, both $p > 0.30$).

Of those males that mated, the time taken prior to commence copulation increased with male age (mean $\pm$ s.e. time to copulation for young males = 49.22 $\pm$ 1.38, intermediate-age males = 67.30 $\pm$ 0.89, old males = 89.03 $\pm$ 2.29; $F_{2,195} = 4.70$, ordered heterogeneity test: $r_s P_c = 0.99$, $p = 0.0001$). However, no other factor contributed to the observed variation in latency time to copulation (mating regime, $F_{2,195} = 0.16$; mating regime $\times$ male age, $F_{4,195} = 1.26$; male body length, $F_{1,195} = 1.39$; female body length, $F_{1,195} = 0.07$; all $p > 0.20$).

The length of time spent *in copula* varied with male mating regime (mean $\pm$ s.e. length of copulation for V males = 85.69 $\pm$ 0.93, SM males = 67.60 $\pm$ 0.40, LM males = 68.71 $\pm$ 1.34; $F_{2,195} = 6.59$, $p = 0.002$). Females mated to V males spent longer in copulation than females mated to either SM or LM males (*post hoc* Student's *t*-test, both $p < 0.05$). There was no difference in the time spent *in copula* between SM and LM males (*post hoc* Student's *t*-test, $p > 0.05$). Moreover, no other parameter in the model explained the observed variation in copulation duration (male age, $F_{1,195} = 2.19$; male age $\times$ mating regime, $F_{4,195} = 1.44$; male body length, $F_{1,195} = 0.07$; female body length, $F_{1,195} = 0.002$; all $p > 0.12$).

(ii) Fecundity

On average only 55.5 $\pm$ 2.9% (mean $\pm$ s.e.) of the mated females commenced oviposition (table 1), but the probability of oviposition was independent of either male mating regime or male age (mating regime, $\chi^2_2 = 4.1$; male age, $\chi^2_2 = 1.32$; male age $\times$ mating regime, $\chi^2_4 = 0.46$; all $p > 0.13$). Moreover, neither female body length ($\chi^2_1 = 0.61$, $p = 0.43$) nor male body length ($\chi^2_1 = 1.48$, $p = 0.22$) influenced the probability of oviposition.

Male age explained a significant amount of the variance in the number of eggs laid by ovipositing females

Figure 2. The mean: (a) total number of eggs; (b) number of fertile eggs; and (c) proportion of fertile eggs laid by females mated to young, intermediate-age and old males. Means and standard errors were obtained from predicted values taken from generalized linear models. See table 2 for sample sizes.

($F_{2,118} = 7.91$, $p = 0.0006$; figure 2a). Females mated to intermediate-age males laid more eggs than females mated to the youngest or oldest males (*post hoc* Student's *t*-test, both $p < 0.05$); however, the number of eggs laid by females mated to young or old males was comparable (*post hoc* Student's *t*-test, $p > 0.05$). Fecundity also correlated positively with female body length ($F_{2,118} = 8.84$, $r = 0.19$, $p = 0.003$), but was independent of male mating regime (male mating regime, $F_{2,118} = 0.28$; male age $\times$ mating regime, $F_{4,118} = 1.11$; both $p > 0.35$).

Table 3. The percentage of females with at least one fertile egg in their clutch following a single mating with a young, intermediate-age or old male from one of three male mating treatments (V, SM or LM).

male mating treatment	male age		
	young	intermediate-age	old
V	45.5	68.8	45.5
SM	26.7	85	69.2
LM	38.9	73.7	58.3

(iii) Fertilization success

The distribution of fertilized eggs was extremely bimodal, with 43% of ovipositing females laying completely infertile clutches (table 3). A nominal logistic regression revealed that females mated to intermediate-age males had the highest probability of fertilizing at least one egg (overall effect of male age: $\chi^2_2 = 17.2$, $p = 0.0002$; *post hoc* Student's *t*-tests on back-transformed data: intermediate-age males $>$ young or old males, $p < 0.01$; young = old males, $p > 0.05$). The same model also revealed that the probability of laying a completely infertile clutch was independent of male mating regime (effect of mating regime, $\chi^2_2 = 2.28$; male age $\times$ mating regime, $\chi^2_4 = 6.15$; both $p > 0.20$).

The total number of fertile eggs laid by ovipositing females was dependent on the age of a female's mate ($F_{2,123} = 7.19$, $p = 0.001$; figure 2b). Females mated to intermediate-age males laid a significantly higher number of fertile eggs than females mated to young or old males (*post hoc* Student's *t*-tests, $p < 0.05$). By contrast, the number of eggs fertilized by young and old mates was comparable (*post hoc* Student's *t*-test, $p > 0.05$). There was no effect of male mating regime on fertilization success (mating regime: $F_{2,123} = 1.79$, $p = 0.17$; male age $\times$ mating regime: $F_{4,123} = 0.96$, $p = 0.43$). The number of fertile eggs was significantly correlated with the number of eggs laid by a female ($F_{1,123} = 272.06$, $r = 0.19$, $p < 0.0001$), but was independent of the duration of copulation ($F_{1,123} = 0.01$, $p = 0.93$).

The proportion of fertile eggs produced by an ovipositing female was dependent on the age of her mate ($\chi^2_2 = 14.44$, $n = 134$, $p = 0.0007$; figure 2c). Intermediate-age males achieved a higher fertilization success than their younger and older counterparts (*post hoc* Student's *t*-test on back-transformed data, both $p < 0.05$); however, there was no difference between the fertilization success of young and old males (*post hoc* Student's *t*-test on back-transformed data, $p > 0.05$). There was no effect of male mating regime on the proportion of eggs fertilized (mating regime, $\chi^2_2 = 1.97$, $n = 134$, $p = 0.37$; male age $\times$ mating regime, $\chi^2_4 = 4.17$, $n = 134$, $p = 0.38$). Further, fertilization success was not related to the duration of copulation ($\chi^2_1 = 0.66$, $n = 134$, $p = 0.42$).

4. DISCUSSION

(a) Overview of results

Our experiments generate three clear findings. First, the mating aggregations revealed that intermediate-age males

obtained the highest proportion of available matings when in competition with younger and older conspecifics. Second, when differences in female fecundity and fertilization success were assessed in the absence of choice, females mated to intermediate-age males were more fecund than females with young or old mates. Moreover, females with intermediate-age mates had higher levels of fertilization success than females mated to younger or older males. Finally, patterns of female fecundity and fertilization success were unrelated to a male's prior mating regime or the age of his sperm.

(b) *Mechanism of choice*

The small mating aggregations did not explore explicitly the relative importance of male interactions or female preferences in determining a male's mating success. That females were equally likely to approach and reject the advances of males regardless of their age, and that males of all ages were likely to approach and reject females, suggest that both sexes are likely to be sampling opposite-sex conspecifics. Males initiated two-thirds of all successful copulations, further indicating that male behaviours are, at least in part, responsible for the observed patterns. Indeed, although males were not permitted a choice of female in this experiment, previous work suggests that they do exhibit preferences, discriminating against mated females in preference for virgins (McNamara *et al.* 2004). However, if male initiation behaviour alone explained the observed mating patterns we would expect that intermediate-age males would initiate copulations more rapidly than young and old males. This was not the case. In the choice trials, older males had the shortest latency times to copulation, but had lower mating success than their intermediate-age counterparts. Moreover, younger males also had low copulation success in choice trials even though they commenced mating most rapidly in mating pairs. This suggests that a mechanism in addition to male initiation behaviour needs to be invoked to explain the variation in male reproductive success.

Intrasexual male competition may have in part precluded female access to the oldest and youngest males in the choice trials; however, several lines of evidence suggest that female hide beetles are also discriminating. While males initiated a large number of copulations, females approached males in one-third of cases and were also able to reject a male by walking away prior to physical contact or throwing him off her back following a copulation attempt. These patterns of female behaviour agree with previous findings on female preferences in the hide beetle (Archer & Elgar 1999). A further point to note is that male hide beetles possess a pheromone gland, a probable secondary sexual trait, which elicits a strong behavioural response in females (Abdel-Kader & Barak 1979). We can only speculate whether females use chemical cues to assess male age, but it is highly probable that male production and dispersal of pheromones play an important role in promoting female preferences, as a preliminary study suggests that the chemical profiles of male pheromones do vary with age (T. M. Jones, unpublished data).

(c) *Male age, sperm age and male mating history*

Our experimental design permits us to begin disentangling the effects of male age, sperm age and male

mating history as potential factors generating the observed declines in fertilization success. Consistent with previous studies, we found that females mated to the oldest males had lower levels of fertilization success than females with intermediate-age mates (Price & Hansen 1998; Jones *et al.* 2000). However, in contrast to earlier work, we found that females mated to the youngest males also suffered a reduction in fertilization success. The experiment found little evidence in the hide beetle to corroborate the prediction that fertilization success correlates with sperm age (Vishwanath & Shannon 1997; Siva-Jothy 2000). Females mated to old V males (who were assumed to have older stored sperm) obtained similar levels of fertilization success to females mated to old SM and LM males (who were assumed to have a greater proportion of younger sperm). Moreover, contrary to expectation (Siva-Jothy 2000), the fertilization success of young males was also consistently lower than intermediate-age males and indeed equivalent to the fertilization success of old males. Our data are also inconsistent with the idea that previous mating history impacts on current fertilization success or fecundity (Wedell *et al.* 2002): there was no effect of male mating regime on overall fertilization success or fecundity.

The declines in fertilization success observed in the young and old age categories of hide beetle may have arisen through differences in the quantity of sperm transferred, which is in turn likely to be related to the specific mode of sperm transfer in the hide beetle. Sperm transfer is typically not 100% efficient, even in internally fertilizing animals. For example, in poultry, only 1% of sperm transferred by males actually reaches a female's sperm storage tubules (Brillard 1993). In the hide beetle, this may be more extreme as males transfer sperm in discrete, tightly woven bundles each containing *ca.* 256 spermatazoa (Al-Taweel & Fox 1983). Any degree of fertilization success therefore requires the transfer of at least one sperm bundle and its associated seminal fluids. Preliminary data suggest that, although copulation duration is unrelated to fertilization success (*cf.* other insect species; Simmons 2001), the amount of sperm transferred by males in each of the three age-classes does vary. In a series of mating trials, young males transferred significantly lower quantities of sperm to a female than intermediate-age and old males; however, old males also transferred less sperm than their intermediate-age rivals (R. Featherston, unpublished data). These data suggest that reduced sperm transfer may explain some of the observed variation in fertilization success. Age-related declines in sperm viability, arising as a consequence of general senescence or the accumulation of germline mutations could exacerbate these effects in the oldest age categories (Kidd *et al.* 2001; Oakes *et al.* 2003; Szczesny *et al.* 2003). Such a mechanism may explain why old and young males suffered comparable declines in fertilization success even though older males transferred significantly more sperm. However, a point to note is that LM males did not suffer from reduced rates of fertilization despite having the highest levels of mating throughout their lifetime and therefore potentially the highest rates of sperm production and germline mutations. Thus, the role of germline mutations in promoting reduced fertilization success may be limited in the hide beetle.

From an evolutionary perspective, it is perhaps unsurprising that sperm age does not determine male fertilization

success in the hide beetle. The mating opportunities of a male are likely to vary throughout his life, particularly if individuals feed and mate on patchy resources. As a result, there may be strong selection pressure among males for an efficient chemical or mechanical means of counter-balancing long periods of mating abstinence (Birkhead & Møller 1998; Kidd *et al.* 2001; Simmons 2001). For example, male hide beetles may resorb older sperm, thus resolving the problem of age-related deterioration of spermatozoa. If so, the assumptions of sperm age made in this study may be inappropriate. Alternatively, males may simply store sperm efficiently. To our knowledge, no study has examined the effects of sperm age in male insects, but females can store sperm for extended periods without appreciable impact on fertilization success (Simmons 2001). Moreover, in the hide beetle, multiply mated females successfully produce offspring up to three months after their last mating (Archer & Elgar 1999) suggesting that sperm storage is highly successful in this species.

(d) Potential costs of mating

For a female, the most obvious immediate gain from mating is the acquisition of sufficient sperm for fertilization. Potential fertilization benefits may be limited by factors such as failed sperm transfer (Micholitsch *et al.* 2000), sperm leakage from the female reproductive tract (Yuval *et al.* 1996) or reduced sperm quality (Crow 1997). In addition to the reduced fertilization success attributed to young and old males in this study, *ca.* 45% of females failed to oviposit. This figure is comparable with the percentage of behaviourally successful matings that result in no sperm transfer in the hide beetle ($n = 21$ matings; R. Featherston, personal communication). Moreover, the probability that a female hide beetle commences oviposition is directly related to the number of matings she receives. Virgin females never oviposit (T. M. Jones, personal observation), while 55% of females commence egg laying after a single mating (this study) and over 85% ($n = 73$ females) of females lay eggs after three matings (K. B. McNamara, personal communication). Hence, the most parsimonious explanation for complete oviposition failure is that a behaviourally successful mating attempt does not always result in sperm transfer (Birkhead 1991; Micholitsch *et al.* 2000). Notably in the hide beetle, failure to oviposit was independent of the age of the female's mate.

We did not assess directly the costs and benefits of copulation; however, the increased fecundity of females paired to intermediate-age males is difficult to interpret using traditional benefits models of sexual selection (Andersson 1994). As male hide beetles offer no appreciable resource to females, and the protein content of seminal fluid is characteristically low (Bownes & Partridge 1987), such increased levels of fecundity are unlikely to be related to the quality of nutrients passed by males during copulation (Hughes *et al.* 2000). Instead, variation in the number of eggs laid may arise through differences in the quality or quantity of accessory cell proteins (ACPs) transferred. An increasing number of studies show that ACPs effect sperm storage and reproductive success, but these are currently confined to a few taxa. For example, in *Drosophila melanogaster*, ACPs stimulate female sperm storage and increase oviposition rates (Chapman *et al.* 1995; Neubaum & Wolfner 1999; Tram & Wolfner 1999).

It is not known whether ACP transfer or production varies with male age. Likewise, the efficacy of ACPs has never been studied in *D. maculatus*. If either the quality or quantity of seminal products is higher in intermediate-age males, this could yield the increased levels of fecundity observed in this study. We are unable to reject the possibility that the increased fecundity of females mated to intermediate-age males arose because females simply invested more heavily in their offspring (Burley 1988). However, as females had an equal probability of commencing oviposition regardless of the age of their mate, to invoke a differential maternal allocation mechanism requires that a female ceases egg laying (rather than never commencing oviposition), if mated to a younger or older male.

(e) Conclusion

In conclusion, the experimental design extends the number of parameters considered by even the most recent models of age-based choice and provides a more realistic insight into the potential mechanisms maintaining male mating success. In contrast to previous studies, we found little evidence to suggest that sperm age (Siva-Jothy 2000) or male mating history (Wedell *et al.* 2002) determined a male's potential reproductive success. Moreover, the observation that females mated to the oldest males suffer reduced levels of fertilization success is inconsistent with the predictions of traditional age-based viability indicator models (Kokko & Lindstrom 1996; Kokko 1997; Proulx *et al.* 2002). Our data instead support the predictions of a number of models that incorporate age-related declines in fertilization success or variation in life-history parameters and propose female discrimination against older males (Hansen & Price 1995, 1999; Kokko 1998; Beck & Powell 2000; Beck *et al.* 2002). The ecology of the hide beetle may be such that potentially choosy females are constrained by their need to obtain sperm from any male that they encounter. However, the experiment provides clear evidence that females can gain direct fitness benefits through mating with intermediate-age males. Such benefits are largely ignored by models of age-related male mating success; however, they may be vital in species such as the hide beetle, for which resources and therefore mates are patchily distributed.

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