Male age, mating probability, and progeny fitness in the bulb mite

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In many species, the accumulation of mutations in the male germline can result in decreased progeny fitness. Consequently, females may evolve preferences for younger partners. Here, we used a promiscuous and relatively long-living bulb mite (Rhizoglyphus robini) to test whether male age affects his progeny fitness. We found that daughters of 4- to 5-week-old males had a 6% lower fecundity than those of 4- to 6-day-old males. Furthermore, we investigated whether male age and female mating status affect the probability of copulation. Male age classes did not differ in motility measured in the presence of females, but older males were less likely to mate within an hour of pairing. We had predicted that females’ reluctance to mate with older males would be revealed by their higher motility after the physical contact between the sexes. However, postcontact female motility was not affected by male age, nor was it a significant predictor of the occurrence of copulation. Apart from male age, mating probability was affected by female mating status, with virgin females copulating more readily than females that had mated multiply before the experiment. The interaction between male age and female mating status was not significant. It took less time for males to locate virgin females, and a higher proportion of virgins mated. Higher male motility in the presence of virgin females suggests that they may use sexual pheromones to attract males, whereas females that have already copulated may avoid the costs of mating by suppressing emission of sexual attractants. Key words: evolution, germline mutations, male age, mate choice, progeny fitness, Rhizoglyphus robini. [Behav Ecol 18:597–601 (2007)]

The traditional view derived from "good genes" models of sexual selection has been that mating with older males should be beneficial for females because old age is a demonstration of a male's high genetic quality that enabled him to survive (reviewed in Brooks and Kemp 2001). However, what really matters for the fitness of a female's progeny is not the quality of her partner but of his gametes. The latter may actually decrease with male age, for example, due to the accumulation of deleterious mutations in his germline (Hansen and Price 1995, 1999; Radwan 2003a). The number of germ-line mutations is likely to increase with age if stem cells continue to divide after an organism has reached sexual maturity, which is the case in many species (Drost and Lee 1995; Hansen and Price 1999). The increasing number of germ cell divisions can be expected to correspond to the accumulation of deleterious mutations in these cells, which in turn will be associated with decreased progeny fitness. Other reasons for a decline of male breeding value with age might include a trade-off between late-age and early-age life history parameters or a decrease in covariance between fitness and fertility with age (Hansen and Price 1995).

In humans, paternal age has been shown to affect the incidence of a number of genetic disorders (Risch et al. 1987; reviewed in Crow 2000). Negative effects of paternal age on offspring fitness have also been shown in other species. Serre and Rohaire (1998) reported a significantly higher neonatal death rate in the progeny of older Norway rat males. Price and Hansen (1998) reported a 3% decrease in larval viability and a 4–6% decrease in male mating ability in the progeny of 34-day-old Drosophila melanogaster males, compared with 2- and 14-day-old ones. In barn swallows, father age accounted for 2–3.5% of the variance in chick body size and feather development (Saino et al. 2002). Jones et al. (2000) observed a higher egg hatching success in lekking sandfly females mated to young and middle aged than to old males, but it is not clear whether this reflected lower quality of zygos sired by old fathers or rather the old males’ lower effectiveness in fertilizing the eggs, for example, due to decreased ability to transfer sperm (Jones et al. 2000). Indeed, in a similar study on the hide beetle, higher hatching success of eggs laid after mating with middle-aged males has been ascribed to the higher fertilization success of their sperm (Jones and Elgar 2004). On the other hand, Pervez et al. (2004) demonstrated a positive effect of male age on egg viability in a predatory ladybird beetle: eggs sired by 20- to 30-day-old males had significantly higher viability than those sired by younger males. However, like in Jones et al. (2000), better egg hatchability might be the matter of fertilization success. As Pervez et al. (2004) show that copulation duration increases with male age, it is possible that older males transfer more sperm and therefore attain higher fertilization success.

In species where older males produce gametes of decreased quality, evolution of females’ preferences toward younger males may be expected. As yet, the evidence for this is scarce. Female discrimination against old males was observed in the lekking sandfly (Jones et al. 2000) and the hide beetle (Jones and Elgar 2004); however, in both systems, females actually preferred middle-aged males to both old and young ones. Female bushcrickets were found to discriminate against older males (Wedell and Ritchie 2004), yet the authors attributed it to age-based differences in the amount of resources provided to a female at mating, rather than to differences in genetic quality. Radwan et al. (2005) found that in the bulb mite, females that had previously copulated were less likely to mate with old than with young males; this difference was not observed for virgin females.

Rhizoglyphus robini (Acari: Acaridae) is well suited to test the effects of male age. It is relatively long living for such a small species, with adult males surviving for up to 2 months (Radwan and Bogacz 2000). The species is highly promiscuous and reproduces continually, which implies that spermatogenesis...
must be intense throughout adulthood and older males must have undergone a large number of germline divisions. Radwan and Bogacz (2000) found that older males transferred fewer sperm, and Radwan et al. (2005) subsequently confirmed that as a consequence, older males lost in sperm competition with younger males. Thus, in this species, multimale mating may be an efficient strategy to avoid fertilization by old males’ sperm (Radwan 2003a). However, the benefits of fertilization by the sperm of younger males remained a matter of speculation in this system (Radwan et al. 2005). The present study was motivated by the need to address this question.

The first aim of the present study was to investigate the fitness consequences of mating with males of different ages. We concentrated on the fecundity of the daughters of old and young males as this major fitness component can be measured precisely and has been shown to be sensitive to the accumulation of deleterious mutations (Radwan 2003b; Radwan et al. 2004). Differences in progeny quality may result from the genetic quality of the parents but also from different investment in eggs by females in response to male quality (Cunningham and Russell 2001; Sheldon 2000) or to nutrients provided by males on mating. Male *R. robinii* do not provide any nuptial gifts and because remating has been shown to be costly for females (Kołodziejczyk and Radwan 2003; Konior et al. 2006), transfer of nutrients in ejaculates seems unlikely.

As any nongenetic effects of father age, affecting maternal investment in eggs, would confound our results, we decided to control for them by measuring the size of eggs laid after mating with young and old males.

The second aim of the study was to collect detailed behavioral data that would allow us to test whether the lower probability of mating by older males (Radwan et al. 2005) is a result of their aging and a consequent decrease in mate securing ability (e.g., because of deteriorated mate searching efficiency) or whether it results from female discrimination against mating with older males. We predicted that aging could result in decreased male motility, thus decreasing male mate searching efficiency and causing higher latency to the first contact between sexes. If, on the other hand, females avoid mating with older males, we predicted higher female motility following her location by older males. Radwan et al. (2005) found that the frequency of mating with older males was significantly decreased only when females had previously mated. Thus, in the present study, we also tested whether the probability of copulation is affected by the interaction between male age and female mating status (virgin vs. mated females).

**METHODS**

**Study animals and rearing conditions**

We used a stock culture derived from a colony of about 200 individuals found on onions in a garden near Cracow, Poland, in 1998, and kept in the laboratory as a large population (about 1000 individuals, subdivided into 6 subpopulations mixed once a month) for about 100 generations before the commencement of this research. The subpopulations were kept in jars 2.5 cm in diameter and 2 cm high, maintained at 22–26 °C, >90% humidity, and fed once a week with a 3:1 mixture of powdered yeast and wheat germ ad libitum. Once a month about a quarter of the food and debris, containing several hundred mites at different stages of development, were transferred to fresh jars. We maintained the same feeding, humidity, and temperature conditions throughout all the experiments described below. We kept individually isolated mites, pairs, and small groups of mites in glass tubes 0.8 cm in diameter and 2 cm high with plaster of Paris bases soaked with water and provided them with food ad libitum.

**Male cohorts**

Both experiments described below involved 2 male cohorts. Young males were taken for experiments 4–8 days after emergence and old males 30–40 days after emergence.

We established the old male cohort 4 weeks in advance of the young male cohort. We obtained each cohort of males by placing 100 females in 5 jars with plaster of Paris bases, 2.5 cm in diameter (20 females per jar), to lay eggs. After 5 days, the eggs began to hatch, and we were providing food regularly to developing larvae. On day 15, we isolated tritonymphs (the last nymphal instar) individually to separate tubes. On day 17, we sexed newly emerged adults and added 2 females to each tube containing a male. In the old male treatment, we kept males with females for 30–40 days and transferred them into new tubes every 10–12 days (before eggs laid by females developed into adults) to avoid confusing them with newly hatched males. Females were replaced if necessary. In the young male treatment, we kept males with females for 4–8 days. As matings occur at the frequency of about 8 per day (Radwan and Siva-Jothy 1996) and previous partners are not discriminated against (Konior et al. 2001), young males had mated on average >30 times before they were used for experiments. Nevertheless, apart from age, male cohorts differed in the number of matings when used in the experiments. However, apart from giving young males opportunity to deplete any sperm reserves they might have had at virginity, we chose not to control for male mating history because in nature male age and the number of his matings are tightly correlated. Thus, any adaptations females might have evolved to avoid fertilization by old males’ sperm may be equally based on male age per se or on his mating history.

**Female mating history**

Females were obtained by isolating tritonymphs and were 3–7 days old when used in the experiments. In the virgin female treatment, we kept females individually in the tubes until the commencement of the experiment. In the nonvirgin female treatment, each female had been kept with a male for 3–7 days and had therefore mated on average >50 times before being used for experiments (see above). Males used in nonvirgin female treatment were obtained from the same set of tritonymphs as the females.

**Progeny fitness**

In the first experiment, we measured fitness consequences of mating with old and young males. Both age classes were paired with virgin females for 2 days. We collected 4–5 eggs from each female, mounted them on a slide, and photographed under a microscope under 200× magnification. We then determined the 2-dimensional area of each egg using AnalySis software. The remaining eggs were left until the larvae emerged, and 5 larvae were then isolated into separate tubes. When they developed into adults, we sexed them, and we randomly picked one daughter from each pair and paired her with a male. The males were obtained from nymphs collected from the stock culture and were 3–5 days postemergence. We replaced the males every 5 days (or earlier if a male died) to avoid effects of occasional male subfertility. We then counted all eggs laid by females for 2 weeks period, which represents about two-third of female reproductive activity (Konior et al. 2001). We did not measure female lifespan because of logistic constraints, but as females’ fecundity is relatively constant throughout most of their reproductive lives, such measure should be a good approximation of their fitness (Konior et al. 2001).
Mating probability

The second experiment was designed to obtain data on male mating ability and female mating preferences. We observed the behavior of 94 pairs: 23 virgin females mated with young males, 24 virgin females mated with old males, 23 nonvirgin females mated with young males, and 24 nonvirgin females mated with old males. Observations were made in glass tubes with bases covered with millimeter-scale paper. Food was evenly dispersed on the paper. We placed a female in a tube first, and after 5 min delay, we measured her motility by counting the cells on the scaled paper she passed within 3 min. Then we added a male to a tube and measured his motility in the same way. If copulation did not commence within 1 h, the observation was stopped and labeled as “no copulation.”

All statistical analyses were performed using Statistica 7 (StatSoft Inc., Tulsa, OK).

RESULTS

The average size of eggs laid by females mated to young males was 3660.4 \( \mu m^2 \), \( n = 89 \); standard error (SE) = 49.1 and of those laid by females mated to old males was 3723.8 \( \mu m^2 \), \( n = 70 \); SE = 53.5. The difference in egg size was not significant (1337 = 0.87, \( P = 0.386 \)). However, we found a significant effect of sire age on the fecundity of his daughters (\( t_{86} = 1.98 \), \( P = 0.048 \)).

We used a logit model implemented in Statistica to analyze the effects of male age, female mating status, and motility of both sexes on the probability of copulation. Both male old age and female prior mating significantly decreased the probability of copulation, but the interaction between these 2 factors was not significant (Table 1). The percentages of old and young males that copulated within 1 h were, respectively, 60.8% and 86.9% when paired with virgin females and 30.4% and 60.8% when paired with nonvirgin females (\( n = 24 \) for both groups of old males, and \( n = 23 \) for both groups of young males). Neither female nor male motility prior to contact affected the probability of copulation (Table 1 and see Table 2 for basic statistics).

We had hypothesized that female resistance to mating would be detectable in her higher motility after her location by a male. However, motility after contact with a male did not significantly predict the occurrence of copulation (likelihood ratio \( X^2 = 2.33, P = 0.136 \)). Neither male age (\( F_{86} = 1.44, P = 0.233 \)) nor female mating status (\( F_{86} = 1.21, P = 0.273 \)); age \( \times \) status interaction: \( F_{86} = 0.89, P = 0.345 \) affected female motility after contact with a male (Table 2).

Male motility, which we assumed to reveal his potential for locating a female, was significantly lower in males paired with nonvirgin females. It was also lower in older males, yet this effect was not significant (Figure 1). Latency to the first contact between male and female was significantly shorter for virgin females and was not affected by male age (Figure 2 and Table 2).

Table 1

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Asymptotic standard error</th>
<th>Wald statistics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept -0.25</td>
<td>0.57</td>
<td>1.98</td>
<td>0.18</td>
</tr>
<tr>
<td>Female motility -0.05</td>
<td>0.06</td>
<td>0.57</td>
<td>0.45</td>
</tr>
<tr>
<td>Male motility 0.02</td>
<td>0.06</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>Male age -0.67</td>
<td>0.25</td>
<td>7.44</td>
<td>0.01</td>
</tr>
<tr>
<td>Female status -0.63</td>
<td>0.25</td>
<td>6.34</td>
<td>0.01</td>
</tr>
<tr>
<td>Male age ( \times ) female status -0.03</td>
<td>0.25</td>
<td>0.01</td>
<td>0.90</td>
</tr>
</tbody>
</table>

DISCUSSION

We found that daughters sired by older males were of lower quality than the daughters of young males. This indicates that the genetic quality of male gametes decreases with age, as expected due to the accumulation of deleterious mutations during germ cell divisions (Hansen and Price 1995). The effect of male age on progeny quality has rarely been tested (reviewed by Radwan 2003a). In an experiment of design similar to ours, Price and Hansen (1998) found a 3% decrease in larval viability and about 4–6% decrease in male mating ability in the progeny of 34-day-old D. melanogaster males, compared with 2- and 14-day-old ones; they found no significant effect of male age on the fecundity of his daughters. In the present study, we additionally controlled for potentially confounding nongenetic effects, that is, the possibility that females invest more resources in eggs fertilized by younger males. Such non-genetic effects should most likely be revealed in increased egg size, although we cannot exclude some more subtle ways in which they could act. The lack of size difference between the eggs fertilized by old and young males suggests that observed fitness advantage of the daughters sired by young males is likely to result from genetic rather than nongenetic effects. Thus, females may gain genetic benefits by avoiding fertilizations by sperm from older males. These benefits are not very high but similar in magnitude to those associated with “good genes” benefits. Moller and Alatalo (1999) have found that 1.5% of the variance in progeny quality is explained by male sexual traits, whereas in our study 1% (\( r^2 = 1.07 \)) of the variance is explained by male age. However, good genes benefits may be overestimated, as a meta-analysis by Moller and Alatalo (1999) is based on studies that do not control for differential maternal investment in eggs, which in mallard was shown to depend on male attractiveness (Cunningham and Russell 2000).

Table 2

<table>
<thead>
<tr>
<th>Age</th>
<th>Status</th>
<th>Female precontact motility</th>
<th>Male motility</th>
<th>Latency to first contact</th>
<th>Female postcontact motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>y</td>
<td>v</td>
<td>4.8 ± 0.7</td>
<td>12.4 ± 1.7</td>
<td>298.8 ± 53.4</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>y</td>
<td>m</td>
<td>4.1 ± 0.9</td>
<td>8.8 ± 1.1</td>
<td>669.8 ± 93.0</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>o</td>
<td>v</td>
<td>5.1 ± 0.8</td>
<td>8.1 ± 1.7</td>
<td>315.9 ± 51.6</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>o</td>
<td>m</td>
<td>5.9 ± 0.8</td>
<td>7.0 ± 1.0</td>
<td>618.7 ± 86.6</td>
<td>3.7 ± 0.6</td>
</tr>
</tbody>
</table>
Females may avoid fertilizations by older males either by evading mating with them or by discriminating against their sperm postinsemination. Radwan et al. (2005) showed that old males achieve lower success in sperm competition than young males. Their results also indicated that older males may be discriminated against, especially by females who have already copulated and thus ensured fertilization of their eggs. The results of the present study confirmed that older males are less likely to mate, but there were some differences compared with results obtained earlier. Radwan et al. (2005) found no significant difference between old and young males in the probability of mating with a virgin female within 90 min of pairing, whereas older males were less likely to mate with females that had already copulated. In one experiment, males were paired with virgins only, and the proportion of males that mated within 90 min was almost identical for both age classes (84% of young males and 86% of old males). In the second experiment, females were paired consecutively with 2 males of both age classes, which enabled testing the effect of female mating status on the probability of mating. In that experiment, more young than old males copulated with both virgin and mated females (88% vs. 74% for virgins and 95% vs. 71% for nonvirgins), but the difference was only significant for nonvirgins (Radwan et al. 2005). In the present study, the difference between young and old males was higher in both female treatments (87% of young males vs. only 61% of old males for virgins and 61% of young males vs. only 30% of old males for nonvirgins). Furthermore, although the difference between young and old males was higher when they were paired with nonvirgin females, the interaction between male age and female mating status was not significant. Thus, the present study did not confirm the suggestion of Radwan et al. (2005) that females’ mating status affects their choosiness with respect to the age of their partners. This might be attributable to differences in experimental design: in the present study, females in the nonvirgin treatment were paired with just emerged males and kept for 3–7 days, so that they could mate repeatedly during this time. In contrast, in Radwan et al. (2005), females were mated consecutively with 2 males of different ages, so that if a female’s first mate was old, the second one was young and vice versa; they were allowed to mate only once with each male. Therefore, females previously mated to old males may have been more willing to remate with younger partners in order to improve their progeny’s genetic quality or to supplement sperm reserves. Very high (95%) remating rate of females previously paired with old males in Radwan et al. (2005) supports this hypothesis.

The overall proportion of pairs that mated was also lower in the present study than in Radwan et al. (2005). This could have resulted simply from shorter observation time in the present study (90 min compared with 90 min in Radwan et al. 2005). Furthermore, shorter observation time might have exaggerated the difference between age classes in the present study. The longer observation time in the former study, by making it easier for less efficient males to start copulation within the observation slot, could have obscured the lower efficiency of old males in achieving copulations.

Our results do not allow us to resolve clearly whether the lower probability of mating by older males results from their worse phenotypic condition and thus lower efficiency in searching for females and mounting them or from female resistance against mating with older males. We predicted that female resistance would be manifested in their higher motility after physical contact occurred, but neither female mating status nor male age significantly affected female motility. Moreover, contrary to our expectations, female motility after the first contact was not a significant predictor of copulation. We cannot exclude the possibility that females discriminated against old males in some other, more subtle manner. On the other hand, we did not find evidence for worsened physical condition in older males: male motility was not significantly affected by age, and male age affected mating probability when male motility was accounted for (Table 1). Thus, we cannot conclude that the lower mating success of older males resulted from their aging rather than from female choice. Alternatively, older males may have had their sperm depleted as a result of larger number of previous matings (see Preston et al. 2001), and although capable of efficient movement and mate searching, they may have been reluctant to mate until they had enough sperm to transfer. This explanation seems plausible given the evidence for a decreased rate of sperm production by older bulb mite males (Radwan and Bogacz 2000).

**Figure 1**
Effects of male age (x axis; young and old) and female mating status (white bars, virgin; gray bars, mated) on male motility (measured as the number of cells on millimeter-scale paper passed within 3 min) after pairing, but before physical contact with the female. Bars represent mean ± SE. The effect of female mating status was significant (ANOVA: $F_{1,87} = 4.618, P = 0.034$), but the effects of male age ($F_{1,87} = 2.625, P = 0.109$) and interaction ($F_{1,87} = 0.631, P = 0.429$) were not.

**Figure 2**
Effects of male age (x axis; young and old) and female mating status (white bars, virgin; gray bars, mated) on the latency (mean ± SE) to the first contact between the sexes. ANOVA—female mating status: $F_{1,86} = 21.426, P > 0.001$; male age: $F_{1,86} = 0.055, P = 0.816$; and interaction $F_{1,86} = 0.219, P = 0.641$.

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*Behavioral Ecology* (white bars, virgin; gray bars, mated) on the latency (mean ± SE) to the first contact between the sexes. ANOVA—female mating status: $F_{1,87} = 4.618, P = 0.034$, but the effects of male age ($F_{1,87} = 2.625, P = 0.109$) and interaction ($F_{1,87} = 0.631, P = 0.429$) were not.
Although we found no clear evidence of female discrimination against old males, we did find several indications that nonvirgin females may be less willing to mate. First, the time to the first contact was shorter for virgin females. Also, male motility before the first contact with the female was significantly higher when he was paired with a virgin. This may indicate that virgin females advertise their presence more than mated females do, making males search for them more vigorously and locate them sooner.

Indeed, bulb mite females use pheromones to attract males (Mizoguchi et al. 2003), although it remains to be tested whether virgin females use them more frequently or in higher concentration. We argue that because of the cost of mating documented in this species (Konior et al. 2003; Konior et al. 2006), females that have ensured an adequate supply of good-quality sperm by mating with young males are selected to suppress the emission of their sex pheromones in order to decrease the number of subsequent matings. This would not compromise their potential for achieving benefits of polyandry (Konior et al. 2001; Koziełska et al. 2004) as such benefits are gained after mating with just a few partners (Yasui 1997) and mating frequency in this species remains high (about 8 matings per day according to Radwan and Siva-Jothy 1996). Alternatively, males may recognize female status using chemical cues and be more attracted to virgins. However, a large degree of sperm mixing coupled with last male sperm precedence (Radwan 1997) makes it unlikely that males benefit from avoiding mating with nonvirgin females. Furthermore, if the costs and benefits of mating were the same for virgin and mated females, there would be no reason for females to signal their mating status. Another possibility, which we cannot exclude on the basis of this experiment, is a male-driven reduction in subsequent mating propensity of their partners. This could involve transferring antiaphrodisiac substances onto females, as observed in some species including butterflies Pieris napi (Andersson et al. 2000), Pieris rapae, and Pieris brassicae (Andersson et al. 2003) and a bumblebee Bombus terrestris (Baer et al. 2001).

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REFERENCES
