ANIMAL BEHAVIOUR, 2007, **74**, 541–548
doi:10.1016/j.anbehav.2006.12.016

Male age does not affect female fitness in a polyandrous beetle, *Callosobruchus maculatus*

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*(Received 13 December 2005; initial acceptance 2 February 2006;
final acceptance 9 December 2006; published online 23 August 2007; MS. number: 8777R)*

Males in different taxa are likely to suffer from a reduction in the quantity and/or quality of their sperm with age. This predicts age-related direct and indirect effects on female fitness. Hence, females may be selected to avoid matings with old males, or to employ alternative mating tactics, such as polyandry, to avoid fertilization by sperm of older males. In contrast, 'viability indicator' models of mate choice predict female preference for old males that have proven their survival ability and signal more reliably. We used a polygamous seed beetle, *Callosobruchus maculatus*, to test for the effects of male age on male mating success and examine the relationship between male age and female fitness, measured as female life span, lifetime fecundity, hatching success of eggs, larval development rate and egg-to-adult survival of offspring. Furthermore, we tested the hypothesis that polyandry may protect females against low numbers of functional sperm produced by old males. We report, contrary to previous findings, that male mating success indeed decreases with male age in this species. However, mating with older males did not in any way compromise female fitness and, consequently, we found no support for the idea that polyandry helps females reduce any costs of mating with older males.

Keywords: *Callosobruchus maculatus*; mating success; multiple mating; seed beetle; senescence; sexual selection

The effect of male age on female fitness and mate choice is a long-standing controversy in the evolutionary studies of mating behaviour (Trivers 1972; Manning 1985; Price & Hansen 1998; Beck & Powell 2000; Jones et al. 2000; Brooks & Kemp 2001; Radwan 2003; Jones & Elgar 2004). Age-related models of mate choice maintain that females should preferentially mate with older males because they have proven survival ability (Trivers 1972; Manning 1985; Kokko & Lindström 1996) and because the reliability of male signalling increases with age (Proulx et al. 2002). However, the quality of male gametes is likely to decline with age as the number of germ cell divisions, which lead to de novo mutations in the male germ line, increases with age (Crow 1993, 1997; Drost & Lee 1995; Hansen & Price 1995; Radwan 2003). Such effects may be particularly important in highly promiscuous species, which produce larger numbers of sperm (Bartosch-Härlid et al. 2003). Thus, females that mate with older males may have a higher chance of being fertilized by gametes of decreased genetic quality, resulting in lower fitness of their offspring. There is some evidence that progeny of older males indeed show reduced survival (neonatal death rate in Norway rats, *Rattus norvegicus*: Serre & Robaire 1998; larval viability in *Drosophila melanogaster*: Price & Hansen 1998; hatching rate in sandflies *Lutzomyia longipalpis*: Jones et al. 2000; see Radwan 2003 for review). Furthermore, older males may suffer from sperm depletion (Wedell et al. 2002; Wedell & Ritchie 2004), especially if the ability to replenish sperm decreases with age (e.g. Radwan & Bogacz 2000). Additionally, in species where sperm is stored for a considerable amount of time, older males will possess older sperm, which may have negative effects on zygote viability (Crow 1997; Siva-Jothy 2000; Reinhardt & Siva-Jothy 2005). Collectively, these arguments suggest that females may be selected to avoid matings

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0003–3472/07/$30.00/0

with older males (Hansen & Price 1995; Jones et al. 2000), or to employ alternative mating tactics, such as polyandry (Radwan 2003; Radwan et al. 2005), to avoid fertilization by sperm of older males.

We examined the effect of male age on female fitness and male mating success in a polyandrous beetle, *Callosobruchus maculatus*. This species has been widely used in recent years to investigate different aspects of male–female coevolution and the evolution of mating systems (e.g. Fox 1993; Savalli & Fox 1999; Crudgington & Siva-Jothy 2000; Eady et al. 2000; Arnqvist et al. 2005; Rönn et al. 2006). Mating in *C. maculatus* is costly to females due to internal injuries inflicted by spiky male genitalia (Crudgington & Siva-Jothy 2000). Such injuries reduce female life span (Crudgington & Siva-Jothy 2000) and lower lifetime fecundity (Edvardsson & Tregenza 2005). Additional costs of mating probably come from toxic seminal compounds, which have been documented in a related bruchid beetle, *Acanthoscelides obtectus* (Das et al. 1980). Jointly, these studies suggest that polyandry can be disadvantageous to females due to the cumulative costs of mating. However, *C. maculatus* females are also likely to enjoy direct benefits of multiple mating. *C. maculatus* males produce large ejaculates that constitute up to 10% of a male's body mass (Savalli & Fox 1998). The size of the spermatophore is known to affect female fecundity (Savalli & Fox 1999; Eady & Brown 2000), and the direction of this effect may depend on male age at mating (Eady & Brown 2000). Females can obtain water and, possibly, nutrients from male ejaculates (Savalli & Fox 1999) and a single mating can increase female life span (Rönn et al. 2006). Another recent study has shown that females are more likely to remate when dehydrated (Edvardsson 2005). Consequently, the relationship between mating rate and female fitness in *C. maculatus* is complex and nonlinear as was shown by Arnqvist et al. (2005).

Several hypotheses suggest that *C. maculatus* females may benefit from, or at least mitigate the costs of, multiple mating by avoiding age-related decline in ejaculate quality (Radwan 2003). First, old males may suffer from sperm depletion, since in this species, like in many other taxa, ejaculate size decreases with the number of matings (Savalli & Fox 1999). The size of the ejaculate, however, is dependent on the time interval between matings (Fox et al. 1995), which suggests that males produce sperm throughout their life. The effect of a decrease in ejaculate size can be amplified if there is an age-related decrease in sperm replenishment (e.g. Radwan & Bogacz 2000). Second, older males may produce subfertile sperm even when capable of mating, if spermatogenic tissue is ageing faster than somatic tissue (Radwan 2003), leading to decreased hatching success. *C. maculatus* is a short-lived but promiscuous species, with both males and females mating multiply during their reproductive cycle. As this species is a short-lived one, a decline in genetic quality of sperm due to mutagenesis may not be severe; however, promiscuity may accelerate ageing of spermatogenic tissue. Two studies suggest that male mating history may have an effect on female life span (Paukku & Kotiaho 2005) as well as female fecundity (Ofuya 1995) in *C. maculatus*. Furthermore, Jones et al. (2000) found reduced hatching success of eggs produced from old males in a similarly short-lived sandfly, *L. longipalpis*. When genetic quality of male gametes declines with age, females may benefit from multiple mating if sperm competitiveness also declines with age. Recent studies indicate that older males indeed lose out to younger males in sperm competition (Schäfer & Uhl 2002; Radwan et al. 2005; but see LaMunyon 2001).

The goal of this study was three-fold: (1) we examined the effect of male age on female fitness, measured as female life span, lifetime fecundity, hatching success, larval development rate, egg-to-adult survival of offspring and total offspring production; (2) we tested the hypothesis that polyandry may protect females against the infertility of older males; and (3) we tested for the effect of male age on male mating success.

METHODS

We used a strain of the seed beetle *C. maculatus* originating from Mali. Beetles were obtained from Peter Credland (University of London) and cultured on black-eyed beans, *Vigna unguiculata*, at 30 ± 0.5°C and 45% (±10%) RH with a 12:12 h light:dark cycle prior to and during the experiment. These beetles are capital breeders, that is they complete their life cycle using only resources acquired during their larval stage (Fox 1993). They are cosmopolitan pests of legume storages, such that laboratory conditions are a good approximation of their natural environment (Fox et al. 2003). The beetle culture used in this study was maintained in our laboratory for 30 generations prior to the experiments. All females used in this experiment were virgin and 1 day post-eclosion. Males were derived from two groups, the young males, which were also virgin and 1 day post-eclosion, and the old males, which were nonvirgin and 5 days post-eclosion. The old males were kept in groups together with nonexperimental females for 4 days (five males and five females in 90-mm petri vials) and as males and females of *C. maculatus* mate multiply during their life span (Ofuya 1995; Arnqvist et al. 2005; Rönn et al. 2006), we expected these males to mate multiply during this period. After 4 days, males were separated and kept individually in 30-mm petri vials for 24 h prior to the experiment. Male mortality after 5 days was between 50% and 70%. We thus ensured that old males used in our experiment were: (1) at the very end of their reproductive cycle; (2) mated multiply to several different females so that any potential effects due to sperm depletion or age-related infertility would be revealed; and (3) passed through viability selection. Such a set-up did not allow testing for the effects of sperm age, but such effects are unlikely to play a role in this system, since males are extremely promiscuous and emerge ready to copulate (Fox et al. 2003). This set-up, however, imitates the natural situation when a newly matured virgin female can be mated either by an old multiply mated male or a recently hatched virgin male.

We used 60 old males and 50 young males to examine the effect of male age on male mating success. Young and old males were treated as described above and randomly paired with virgin females. Pairs were introduced to each other in 30-mm petri vials and their behaviour was