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Age-based female preference in the fruit fly *Drosophila pseudoobscura*

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With respect to mate choice, females of many species discriminate between males on the basis of age. The adaptive significance of age-based mate choice is unclear, with various hypotheses making conflicting predictions. We examined the possibility of female preference in the fruit fly *Drosophila pseudoobscura*, a species where females gain no significant nutritional benefits from mating. Females were shown to prefer to mate with old males in two-male choice experiments and accepted old males faster than young males in single-male tests. Females mated to old males had a higher fecundity, possibly related to a transfer of more sperm and/or a larger volume of accessory gland proteins during the longer copulation durations experienced with old males, although these possibilities were not directly tested. Hence, females appear to derive direct benefits from preferring to mate with older males. In contrast, there was no evidence of mate preference for the sons of either old or young males when matched for age, indicating that the mating advantage of old males is not passed to their sons. These sets of experiments suggest that in *D. pseudoobscura* the benefit of age-based preference to females is increased fecundity due to greater investment per mating by older males.

Keywords: age; *Drosophila pseudoobscura*; ejaculate; female choice; indirect benefits; sperm

When females receive only sperm from their mates, they may choose mates on the basis of the genetic quality or attractiveness they confer to their offspring (Anderson 1994). However, the genetic advantages of these choices are expressed only in the next generation, making choices based on these indirect benefits less likely to evolve than those based on direct benefits (e.g. food, access to territory, parental care; Kirkpatrick & Barton 1997; Arnqvist & Nilsson 2000; Brooks & Kemp 2001). Although mate preferences have often been demonstrated in animals (Anderson 1994), determining the benefits to females that result from the preferences is far more challenging, particularly if they are indirect. In addition, studies of sexual selection often consider the influence of only one component on fitness, for example, female choice, whereas the simultaneous effects of male–male competition are often ignored. The potential problems of this approach are illustrated by work on the cockroach *Nauphoeta cinerea* (Moore & Moore 1999). Here female choice operates in one direction and male–male competition in another; hence both processes need to be considered to make inferences about how sexual selection operates. There is no a priori reason to expect male–male competition and female choice to act in a reinforcing manner (Moore & Moore 1999; Bonduriansky & Rowe 2003).

Male age is a trait that has received a lot of attention as a potential cue that females might use to derive both direct and indirect benefits (Trivers 1972; Hansen & Price 1995; Kokko & Lindstrom 1996). Theory suggests that old males should be favoured due to their proven survival ability with only the fittest males able to survive to old age, ensuring a higher average genetic quality (Trivers 1972; Brooks & Kemp 2001). Simply reaching old age is therefore a reliable way of displaying both genetic superiority in current environmental conditions and lack of mutations accumulated at the prezygotic stage that could reduce survival (Manning 1985). This hypothesis is supported by empirical evidence in beetles (Conner 1989; Pervez & Richmond 2004), field crickets (Zuk 1988) and warblers (Hasselquist et al. 1996) for example, but the reverse has also been found (e.g. bushcrickets, Ritchie et al.

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1995; and sand flies, Jones et al. 2000). Hansen & Price (1995) suggest three main reasons that an individual's fitness decreases with age. First, the older the male, the greater the possibility of accumulating mutations in the germline that could offset the advantages of genes for longevity (Crow 1993). Second, in continually evolving populations with long generation periods, younger males would be preferred due to more recent selection acting on their parents, making them better adapted to current environmental conditions. Third and importantly, with regard to resource allocation, males that have invested heavily in survival and fertility at a younger age may have a lower viability and residual reproductive value when old as a result of negative genetic correlations between early and late fitness components (Cordts & Partridge 1996). However, models incorporating resource allocation and genetic variation suggest that there could still be positive correlations between early and late fecundity and survival in many circumstances (e.g. Kokko 1998).

In species where females receive only sperm from males, females should also pay attention to male age to maximize the direct benefits from mate choice in terms of increased fertility. Sperm quality often declines with male age due to accumulated oxidative and thermodynamic damage resulting in reduced zygote viability (reviewed in Reinhardt & Siva-Jothy 2005) or increased mutation load in offspring (Radwan 2003). In these situations females should avoid mating with older males to maximize their fertility (e.g. Jones et al. 2000). On the other hand, older males may also have accumulated increased sperm reserves and/or increased their investment per mating due to low residual reproductive value (Roff 1992). In this case, females should prefer older males because this may result in increased fertility levels.

Although direct advantages to female choice are often more easy to examine, the only way to fully evaluate the possibility of both direct and indirect advantages to females by mating with a male of a specific age is to examine the effects of paternal age in both the current and the subsequent generations (Brooks & Kemp 2001). Indirect benefits can be achieved through either the passing of good genes to offspring (Kirkpatrick & Ryan 1991; Anderson 1994), thus ensuring more viable sons and daughters, or the heritability of male attractiveness (Fisher 1930), with more attractive fathers siring more attractive sons (e.g. Weatherhead & Robertson 1979). Old males passing damaged sperm or sperm carrying a higher mutational load can result in offspring of lower fitness (Radwan 2003). This may be revealed in terms of reduced viability (direct costs) and/or production of offspring with lower reproductive success. For example, in *Drosophila melanogaster*, the mating ability of offspring sired by older males is reduced compared to offspring of younger fathers (Price & Hansen 1998). To elucidate the advantage to females of selecting males on the basis of age, the fitness consequences must therefore be examined not only directly on female fecundity but also in their offspring. Previous experiments examining the possibility of indirect benefits to age-based indicator mechanisms have either found no significant preference for male age (Price & Hansen 1998; Martin et al. 2003) or used variation in egg hatch rate as the sole measure of offspring fitness (Jones et al. 2000).

In this study we investigated whether females of the fruit fly *Drosophila pseudoobscura* show preference for males of specific ages and whether choosy females derive increased fecundity (a direct benefit) and/or produce sons with higher mating success (an indirect genetic benefit). Preference is examined both in two-male trials, where male–male competition and female choice can occur simultaneously, and in single-male trials with only female preference operating. Throughout these experiments we define mate preference as (1) females consistently mating with one group of males over the other (two-male trials) or (2) females consistently having a shorter time to copulation (mating speed) with males of one group when presented alone (single-male trials). These are standard techniques for measuring female mate preference in *Drosophila* species (Speith 1974; Noor 1996; Barth et al. 1997; Basso da Silva & Valente 2000), although we do not imply that female preference is independent of male behaviour and other traits (Eberhard 1996). This protocol allows us to compare female preference and fitness both with and without the influence of male–male competition. *Drosophila pseudoobscura* is an appropriate species for this type of experiment because male ejaculates do not provide any significant nutritional benefits to the female (Snook & Markow 1996), females largely determine whether copulation will take place (Merrell 1949; Ehrman & Parsons 1981) and females are likely to encounter males of varying ages in the wild (Markow & Richer 1992).

METHODS

The study population of *D. pseudoobscura* was derived from a natural population collected at Show Low, Arizona, U.S.A. in August 2004 and has consistently been reared in 5-week generation cycles as a large outbred population ($N > 1600$) since that date. The population is maintained at 23°C under a 14:10 h light:dark period (lights coming on at 1000 hours GMT) in standard *Drosophila* vials (25×75 mm) on a medium of rolled oats, brown sugar, dried yeast, agar, nipagin and water (see Shorrocks 1972).

Flies were collected and segregated by sex within 18 h of eclosion to ensure virginity (Greenspan 1997). Flies were sexed and transferred to fresh food in sex-specific vials of approximately 20 flies per vial. We transferred flies with a mouth pooter and did not anaesthetize them as this can alter copulation latency (Barron 2000). Female flies were moved onto fresh food every 3 days throughout their lives. All experiments were carried out between 1100 and 1400 hours GMT at 21.5 ± 0.5°C to prevent circadian and temperature differences altering the behaviour of the flies (Chapman 1998).

Two-male Tests

Male flies were aged 14 days (old) or 2 days (young). Approximately 30% of male flies survive to 14 days in nature (8% daily mortality; Dobzhansky & Wright 1943)