

Thus these studies in *D. ananassae* suggests that females of *D. ananassae* discriminate males on the basis of age and older males had greater reproductive success over young or middle age males, which is found to be independent from influence of inversion system.

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Male age effects on fitness are independent of inversion system in *Drosophila ananassae* (Diptera: Drosophilidae).

Prathibha, M.*, S.C. Jayaramu, and M.S. Krishna. Department of Zoology, Yuvaraja's College, University of Mysore, Mysore -570006, Karnataka, India.

*Corresponding Author, Email: prathibhajayaramu@gmail.com, Dr. S.C. Jayaramu: jayaramu21@gmail.com, Dr. M.S. Krishna: drosokrish@gmail.com

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 and Lindstrom, 1996; Kokko 1997). Such models argue that females use age as reliable signal of male quality and that, by mating with older males, they gain indirect benefits through the production of higher-quality offspring. Females in many animal species have mating preferences based on male age. Brooks and Kemp (2001) evaluated the conditions under which preferences for older or younger males are expected to evolve and provided several empirical examples of age-based mate choice across diverse group of taxa. Age based mate choice is often linked to the kinds of reproductive benefits females derive. For example, by choosing males on the basis of age females may obtain direct benefits, such as increased fecundity or hatching success of offspring (Jones and Elgar, 2004; Zuk, 1988), or indirect benefits, such as increased offspring survivorship (Conner, 1989; Jones *et al.*, 2007).

Female mate choice is an important feature of most animal mating systems, but the benefits the females derive from their mating preferences often remain obscure (Andersson, 1994). In species in which males provide material benefits to females or their offspring (*e.g.*, food, shelter, and parental care), females can obtain direct fitness benefits by preferentially mating with those males most likely to invest materially in females or their offspring.

A series of verbal models (Trivers, 1972; Manning, 1985, 1989) predict that, given a choice, females should prefer older mates. As old males have proven survival ability, choosy females may gain indirect benefits from their choice of an old mate through the production of higher quality offspring. However, testing of indirect benefit must require the next generation and empirical evidence for indirect genetic benefit.

Therefore, the present study has been undertaken in *D. ananassae* which is a cosmopolitan domestic species. Males of *D. ananassae* do not show parental care and only supply sperm during copulation. In addition to this, this species shows a high degree of chromosomal variability (Singh, 1982). It is known that inversion polymorphism in the genus *Drosophila* is of frequent occurrence and has adaptive function (Da Cunha, 1960; Dobzhansky, 1970).

In contrast to direct benefit, whether females also gain a net indirect benefit from that choice of mates is poorly understood. Therefore much attention has been diverted at good genes' indirect benefits, because their magnitude is key to determining the net fitness effects of mate choice, especially in systems in which direct benefits are weak or lacking and indirect costs exist (*e.g.*, *D. melanogaster*). Contrasting theoretical analyses have suggested that good genes' indirect benefits are capable of overcoming direct costs of mate choice (Hansen and Price, 1995; Kokko, 1998) or that direct selection on mate choice will commonly overwhelm any indirect benefits (Beck and Powell, 2000). Most studies of age based female mate choice were concentrated on direct benefits, but females also gain indirect benefits from such mating. Therefore, more studies are required to test good gene model of age based female mate choice especially in systems in which direct benefits are weak or lacking and indirect costs exist. Further, studies have also found females of species are selective in their mates even in species in which males provide no material resources to females beyond sperm, suggesting that females derive indirect genetic benefits by mating selectively (Andersson, 1994; Jennions and Petrie, 1997). So we used monomorphic and polymorphic strains of *D. ananassae* in order to study the role of age and inversion on fitness.

Materials and Methods

Experimental stock:

Monomorphic (inversion free) and polymorphic (with inversion) strains of *D. ananassae* were established from already established (Jayaramu, 2009; Prathibha and Krishna 2010) out-bred populations collected at semi-domestic localities of Mysore, Karnataka, India. To study polytene chromosomes and to screen for the presence of inversion, the larvae were dissected in 0.7% sodium chloride solution. These salivary glands were fixed in 1N HCL for five minutes. The stained glands were individually placed on slides with two drops of 45% acetic acid. Then salivary glands were squashed by placing a clean cover glass and by applying uniform pressure. This achieves uniform spreading of the chromosomes. After squashing, edges of the cover slip were sealed with paraffin lanolin mixture. The observation of slides was done under Leitz Ortholux II scientific and clinical microscope both at low (10×) and high (45×) magnification. If inversion loop is present in the larvae then the mother of those larvae was treated as polymorphic. If there is no inversion loop in the larvae then the mother of those larvae was treated as monomorphic. Presence or absence of inversions was checked for five generations. Like this polymorphic and monomorphic strains were established. These experimental stocks were maintained at $21 \pm 1^\circ\text{C}$ at relative humidity of 70% using 12:12 L: D cycle. At the 5th generation after testing the inversion, virgin females and bachelor males were isolated within 3 hrs of their eclosion separately from monomorphic and polymorphic strains and aged as required and cultured using same laboratory condition until they were used in the experiments.

Selection of male age classes:

Before assigning male age classes we studied the longevity of males in monomorphic and polymorphic strains of *D. ananassae* by individually transferring unmated males into a vial containing wheat cream agar medium once a week and maintaining them in the same condition. This process was continued until their death and longevity was recorded. A total of 50 replicates were made for each of monomorphic and polymorphic strains and mean longevity data showed 60 ± 2 for monomorphic and 63 ± 2 for polymorphic strain. Since mean longevity of *D. ananassae* ranges from 60-65 days we assigned days for young, middle, and old aged males as follows [Young age male (2-3 days), middle age male (24-25 days), and old age male (46-47 days)]. In addition to this, we also collected 5-6 day old virgin female from the respective stocks to use in the present experiment.

Fitness evaluation in monomorphic and polymorphic strains:

We used unmated young, middle, and old aged males and 5-6 day old virgin females of monomorphic and polymorphic strains to study fitness and longevity. A female along with a male (young/middle/old age) were individually transferred into an Elens-Wattiaux mating chamber and observed for 1 hr. Pairs unmated within 1 hr were discarded. All the mated female by each male were individually transferred to a new vial once in 24 hr until their death to study fecundity (number of eggs laid), fertility (progeny production), and longevity. After counting the eggs as above, eggs (100) were taken randomly using spatula to study egg-larval hatchability and were seeded separately for each of the three male age classes in a small petri dish containing wheat cream agar medium. Number of 1st instar larvae emerged from above eggs was counted to account for percentage of egg-larval hatchability. For studying larval-adult viability, 100 1st instar larvae were collected as above and were placed in a petri dish, and the number of adult flies emerged from these larvae were also counted, to account for percentage of larval-adult viability.

Statistical analysis:

A total of 50 trials were used separately for each of the three male age classes. Two-way ANOVA followed by Tukey's Honest *post hoc* test (Tukey's test) was carried out on data of fecundity, fertility, longevity, egg-larval hatchability, larval-adult viability using SPSS 10.0 Programme. Experiments were done separately for both monomorphic and polymorphic strains.

Results

Female fitness:

Females mated to old male had significantly greater number of eggs and progeny number than female mated to young males. These results were found to be similar in both monomorphic and polymorphic strains (Figure 1). These data subjected to one-way ANOVA followed by Tukey's *post hoc* test showed significant variation between male age classes, and Tukey's test found that females mated to old males had significantly greater fecundity, fertility, egg-larval hatchability, and larval-adult viability than those of female mated to either young or middle aged males.

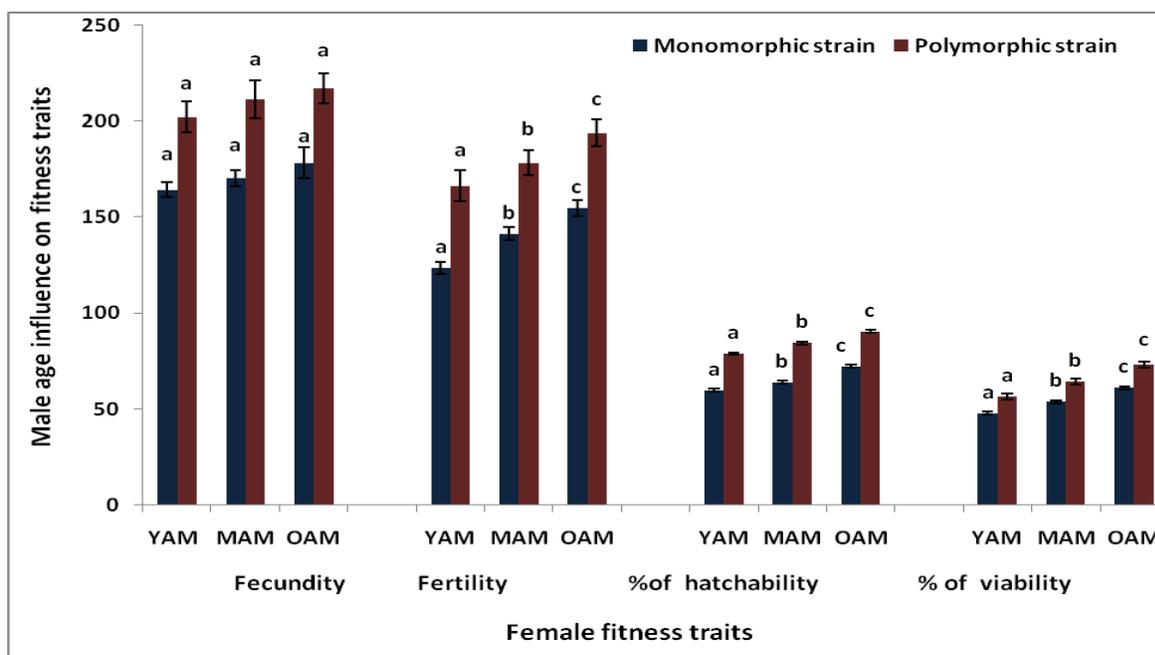


Figure 1. Male age influence on fecundity, fertility, percent of egg hatchability, larva- adult viability in monomorphic and polymorphic strains of *D. ananassae*. (Values are mean \pm SE).

YAM-Young age male (2-3 days), MAM- middle age male (24-25 days), OAM-Old age male (46-47 days); Different letter on bar graph indicates significant variation by Tukey's test, $df=1, 147$. [F-Values of female mated to different male age classes: for fecundity: monomorphic $F=1.51^{NS}$; polymorphic $F=0.76^{NS}$; for fertility: monomorphic $F=19.25$; $P<0.001$; polymorphic $F=3.67$; $P<0.05$; for % of egg hatchability: monomorphic $F=55.18$; $P<0.001$; polymorphic $F=57.46$; $P<0.001$ for % of larva-adult viability: monomorphic $F=70.97$; $P<0.001$; polymorphic $F=34.46$, $P<0.001$].

Figure 1 shows that in *D. ananassae* females mated with old aged males had significantly greater fecundity, fertility, percent of egg-larval hatchability, percent of larval-adult viability, than female mated with young or middle aged males, confirming greater reproductive success of old aged males (Singh 1985a, b; Nicholas *et al.*, 2008a, b; Singh 1989). The result was found to be similar in both monomorphic and polymorphic strains. This could be attributable to longer copulation duration and greater male mating ability. The difference in performance of males of different age classes should be exclusively due to differences in male quality that are attributable to male age. Further experimental design used here eliminated potential influence of female age by mating 5-6 day old female to males of different age classes.

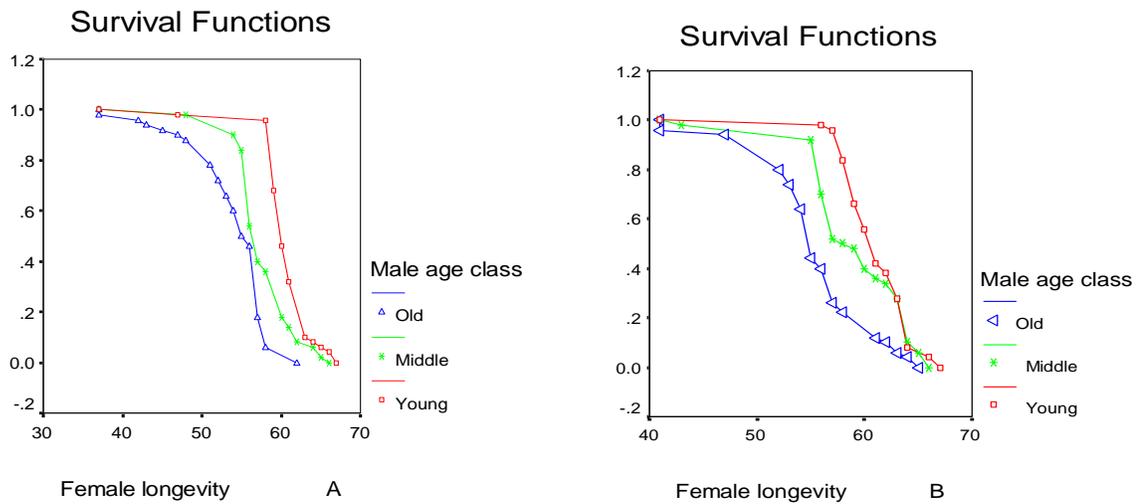


Figure 2. Survival curve of female longevity of *D. ananassae* (A: Monomorphic strain; B: Polymorphic strain).

Table 1. Over all tests of the equality of survival times of monomorphic and polymorphic strains of *D. ananassae*.

Strains	Test Statistics for Equality of Survival Distributions for AGES of Monomorphic and polymorphic strains			
		Statistics	df	Significance
Monomorphic strain	Log Rank	63.37	2	.000
	Breslow	67.36	2	.000
	Tarone-Ware	67.16	2	.000
Polymorphic strain	Log Rank	34.37	2	.000
	Breslow	48.63	2	.000
	Tarone-Ware	42.29	2	.000

Female longevity:

Figure-2 and Table 1 show average longevity of females mated to males of different age classes in both monomorphic and polymorphic strains. It reveals that females mated to young males lived longest followed by females mated to middle aged males, and females mated to old aged males lived shortest. Survival curve analysis showed that female mated with old male survived shorter than female mated with young or

middle aged males (Figure 2 and Table 1).

Figure 2 and Table 1 shows that in *D. ananassae* in both monomorphic and polymorphic strains, female mated with old aged male lived significantly shorter period than female mated with young or middle aged males. This suggests significant influence of male age on female longevity, too. It is not known whether accessory gland secretion that is transferred to female during copulation has carried greater harmful effect with increasing male age or the delayed mating of males up to 46

days might have caused carrying greater harmful effect in their ejaculate and form the cause for reduction in female longevity or not. However, these causes have not been tested presently.

Studies in *Drosophila* have also pointed out that during copulation, in addition to sperm, the seminal fluid contains a cocktail of accessory gland proteins that change the post copulation physiological behavior of the female in various ways. Thereby it has been demonstrated that 1) reduce female receptivity to courting males, 2) increase the egg production rate, and 3) decrease female life span. It has been convincingly demonstrated that the reduction in lifespan resulting from exposure to males is a consequence of harmful effects of both male courtship and seminal fluid (Partridge *et al.*, 1987; Chapman *et al.*, 1995; Vahed, 1998; Long and Pischedda, 2005; Nicholas *et al.*, 2008a, b; Bretman *et al.*, 2009; Wigby *et al.*, 2009).

Polymorphic strain showed greater fitness than monomorphic strain. This confirms the earlier studies of greater fitness of polymorphic strain over monomorphic strain (Spiess, 1970; Singh, 1985a, b; Singh and Chatterjee, 1988; Singh, 1989; Singh and Som, 2001). Even though the polymorphic strain showed greater fitness over monomorphic strain, the inversion system does not affect the age based fitness.

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