feeding rates between 24 and 148 h of larval development in the *mesophragmatica* group (Del Pino and Godoy-Herrera, 1999). In short, investigations on structure of behaviors deal with ecological resources as food may provide indications on evolution of behavior and brain of *Drosophila* larvae.

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Male age effect on fitness is independent of inversion system in *Drosophila ananassae.*

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Abstract

Male age influence on fecundity and fertility has been studied in monomorphic (inversion free) and polymorphic (with inversion) strains of *Drosophila ananassae*. It was noticed that in both monomorphic and polymorphic strains, females mated to old males showed greater fecundity and fertility than females mated to young or middle aged males. Thus, in *D. ananassae* male age effect on fitness is independent of inversion system. Key Words: *Drosophila ananassae,* monomorphic, polymorphic, male age, fitness trait.

Introduction

In studies of sexual selection evolutionary response to selection also depends on the amount of genetic variation present in the population of a given species. The genetic variation could be a consequence of either point mutations or due to gross changes in the karyotype. Karyotypic changes are brought about due to either numerical (ploidy) or structural (chromosomal) aberrations. Numerical changes are found mostly in plants and structural changes are common in animals. Structural changes include deletions, duplications, inversions, and translocations. Although all these four kinds of aberrations are of common occurrence in the animal kingdom, all groups of organisms do not have all four kinds of them. According to White (1977) chromosomal rearrangements have played a major role in evolution and the phenomenon has occurred many times in the evolutionary history so as to produce new variants.

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 and Price, 1995; Kokko and Lindstrom, 1996). Theory suggests that males should be favored 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 and 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 and Richmond, 2004), field crickets (Zuk, 1988), and warblers (Hasselquist *et al.*, 1996). But the reverse has also been found in bush crickets (Ritchie *et al.*, 1995) and in sand flies, (Jones *et al.*, 2000). Hansen and 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 germ line 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 and 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 (Kokko, 1998). Further, very few studies have been carried out to study male age influence on offspring qualities (Pervez *et al.*, 2004; Paukku and Kotiaho, 2005; Prokop *et al.*, 2007). Even in these studies, they have found both negative and positive influence of male age on offspring qualities. The difference in conclusions of these models is a probable result of their contrary approaches towards the change in physiological state of males with age and the existence of life history tradeoffs. Unless more species and genera are involved, it is difficult to understand the concept. Hence more studies are needed in this regard. To test these, model species of *Drosophila* as they do not show parental care is more suitable.

D. ananassae has been selected as the experimental model in the present investigation, because of its following characteristics. It is a cosmopolitan domestic species belonging to the *melanogaster* group of *ananassae* subgroup and *ananassae* species complex (Bock and Wheeler, 1972). This species occupies a unique status in the whole of genus *Drosophila* due to certain peculiarities in its genetical behaviour (Singh, 1985a, b). Presence of male crossing over, high levels of inversion polymorphism, and high mutability are the features which make it useful for certain genetic studies. Therefore, the present study has been undertaken in *D. ananassae* to study the role of inversion system in male age effect of mating activity and female fitness.

Materials and Methods

Monomorphic (inversion free) and polymorphic (with inversion) strains of *D. ananassae* were established from out-bred populations collected at semi domestic localities of Mysore, Karnataka, India have been used to study male age effect on mating success and female fitness. To study polytene chromosomes and to screen for the presence of inversions, 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 5 generations. Like this polymorphic and monomorphic strains were established. These experimental stocks were maintained at 21±1°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 hr of their eclosion separately from monomorphic and polymorphic strains, were aged as required, and were cultured using the 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 transferring individually unmated male into a vial containing wheat cream agar medium once in a week and maintained 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± days for monomorphic and 63±2 days 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 days old virgin females from the respective stocks, and these were used in the present experiment.

Male age influence on fecundity and fertility in monomorphic and polymorphic strains

We used unmated young, middle, and old aged males and 5-6 days old virgin females of monomorphic and polymorphic strains to study male age influence on fitness. A female along with a male (young/middle/old age) were individually transferred into an Elens-Wattiaux mating chamber and observed for 1 hr. Any pair unmated within 1 hr was discarded. All the mated females by each male were individually transferred to a new vial once in 24 hr until their death to study fecundity and fertility. Total number of eggs and progeny produced were recorded. Experiments were done separately for both monomorphic and polymorphic strains.

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 using SPSS 10.0 Programme.

Results and Discussion

Male age influence on fitness

Figures 1a and 2a, and Tables 1b and 2b, show mean values of fecundity and fertility data of both monomorphic and polymorphic strains. Females mated to old males had greater fecundity and fertility than females mated with either middle or young males. Two-way ANOVA followed by Tukey's honest post-hoc test carried out on above data showed significant variation between male age classes between strains.

The concept of fitness has played a key role in the development of evolutionary biology (Haymer and Hartl, 1982). Fitness in general is a property that can be attributed to an individual, a genotype, or a species and refers to the ability of an organism to leave surviving offspring (Ayala, 1965). Fecundity and productivity are the two intrinsic factors contributing to fitness (Soliman, 1973). Evolutionary biologists relate certain biological phenomenon with the measurement of fitness. Population geneticists call these biological phenomena "components of fitness" (Dobzhansky *et al.*, 1963; Marinkovic, 1967).

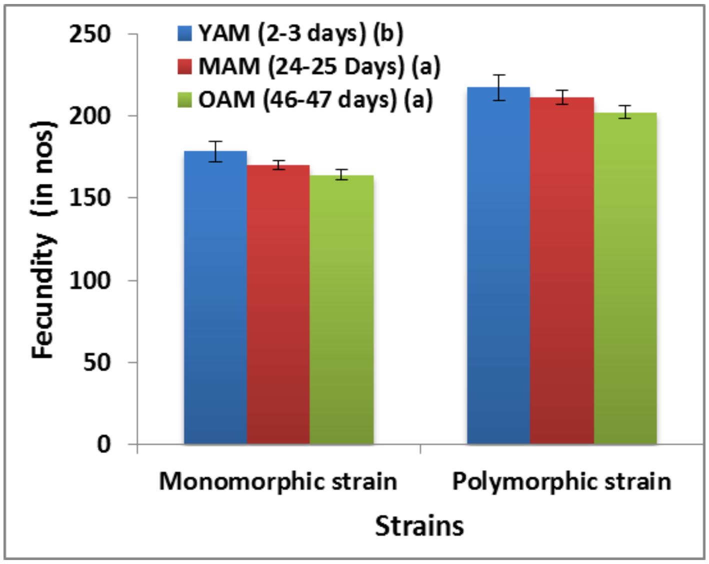

Figure 1a. Male age influence on fecundity in monomorphic and polymorphic strains of *D. ananassae* (Values are mean ± SE).

These fitness characters are influenced by the factors such as species, genotype, body size, age, and by her mate or male's effects as well as by environmental factors such as crowding and temperature (Robertson, 1957; Markow and Akney, 1984; Hoffmann and Harshman, 1985; Partridge *et al.*, 1986; David *et al.*, 1983; David, 1988). In the present investigation it was noticed that female mated with old age male had significantly greater fecundity and fertility than female mated with either young or middle aged males (Figure 1a and 2a, and Table 1b and 2b).

It is not known whether reaching old age should have trading with other fitness traits or it should have positively related to other fitness traits; this might have a bearing on sexual selection. In the present study we have not noticed trade-off between early and late effect of male age on fitness traits in fecundity and fertility of females mated with early and late age males in *D. ananassae*. In other words in *D. ananassae* females preference for old age male could be due to obtaining direct fitness advantage rather than tradeoff between age

Table 1a. Two Way ANOVA of fecundity of female mated to males of different age classes in monomorphic and polymorphic strains of *D. ananassae.*

Fecundity	Source	Type III Sum of Squares	df	Mean Square	F-Values
Fecundity (in no)	Strains	116308.830	1	116308.830	43.07**
	Male age	10569.247	2	5284.623	0.143^{NS}
	Strains*Male age	145.500	2	72.750	.027*
	Error	793862.220	294	2700.212	
	Total	11819775	300		

* Significant at 0.05 level, ** Significant at 0.0001 level, NS- Non significant

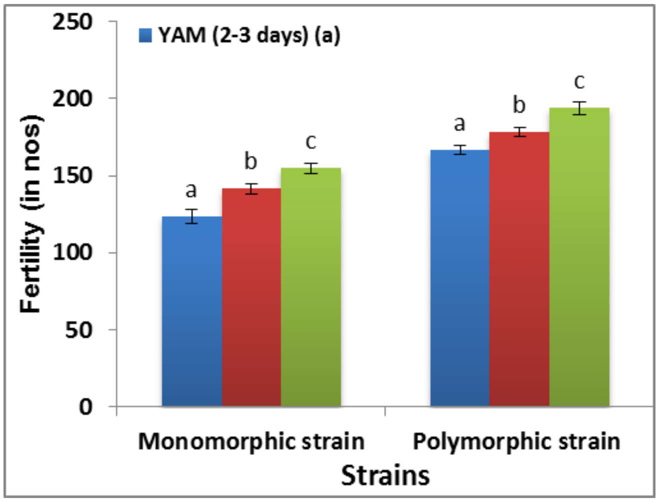

Figure 2a. Male age influence on fertility in monomorphic and polymorphic strains of *D. ananassae* (Values are mean ± SE).

Table 2b. Two Way ANOVA of fertility of female mated to males of different age classes in monomorphic and polymorphic strains of *D. ananassae.*

Fecundity	Source	Type III Sum of Squares	df	Mean Square	F-Values
Fecundity (in no)	Strains	117572.403	1	117572.403	72.770**
	Male age	43162.747	2	21581.373	13.358**
	Strains*Male age	482.027	2	241.013	.861^{NS}
	Error	475005.420	294	1615.665	
	Total	8296715.000	300		

* Significant at 0.05 level, ** Significant at 0.0001 level, NS- Non significant

and fitness traits. This is in contrast to the model of Hansen and Price (1995) mutation accumulation theory and antagonistic pleiotropy theory (Charlesworth, 2001; Partridge and Gems, 2002). These models and theories are based on the idea that mutations that have deleterious effects only later in life after the normal breeding age will have a greater chance of persisting and accumulating in the population in the face of natural selection. The antagonistic pleiotropy theory suggests in addition that the late acting deleterious genes may have beneficial effects early in life and are thus favored by selection and will be actively accumulated in populations despite their deleterious effects late in life. However, evidence is accumulating on age-dependent expression of genes affecting reproduction. For example, about half of the genes down regulated with age in *Drosophila melanogaster* are linked to reproduction (Girardot *et al.*, 2006), and female fecundity seems to be determined by different loci at different ages, with no genetic correlation between early and late age fecundities (Leips *et al.*, 2006). In our study we also found that polymorphic strains had greater reproductive success over monomorphic strains (Singh, 1989; Singh and Chatterjee, 1988; Singh and Som, 2001). This confirms the earlier studies of greater fitness of polymorphic strains over monomorphic strains (Dobzhansky and Levene, 1951; Jayaramu, 2009). Thus, these studies in *D. ananassae* suggest 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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Reproductive advantage of middle aged females in monomorphic and polymorphic strains of *Drosophila ananassae.*

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Abstract

Monomorphic (inversion free) and polymorphic (with inversion) strains of *Drosophila ananassae* established from out-bred populations collected at Mysore has been used to study female age influence on mating success, mating latency, courtship activities, copulation duration, and female wing length. It was noticed that polymorphic strains had greater mating success, mated faster, performed greater courtship