Discussion

M M Hills- A total of 3163 flies were recorded, 10 species were found (Table 1). The flies were found in summer season and flies tend to be increased in bottom region of the hill. Interestingly, flies were decreased with increasing altitude. *D. melanogaster*, *D. neonasuta*, *phorticella striata* and *D. nasuta* could be assigned as dominant species, as they are available at all the altitudes during 2011. The reason for this may be easily understood if we observe the quantity of the species if dominance at each altitude. When population sizes are relatively small and fluctuate, there is a possibility of populations going extinct due to demographic and environment stochasticity. Thus, from the present study the distribution of species is uneven in space and time.

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Male age influence on pre-adult fitness in *Drosophila ananassae*.

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Introduction

In insects investigation on mate choice have shown effects of body size on mating events and reproduction (Hegde and Krishna, 1997). Apart from body size, the age of mating individuals also plays an important role in mate choice and reproduction (Pervez, *et al.*, 2004). It was widely held that females should prefer to mate with older males in species where males provide only sperm to females, because viability selection leads to older males of higher genotype quality than young males (Trivers, 1972; Manning, 1985; Andersson, 1994). In support of this hypothesis, a simulation model revealed a strong preference for older males under different eco conditions (Kokko and Lindstrom, 1996). Several empirical studies have suggested that females do indeed prefer to mate with older males for qualitative and quantitative progeny production (Zuk, 1988; Manning, 1989; Simmons and Zuk, 1992; Simmons, 1995). However, some have argued that males of young and intermediate ages are better mates, as they have the highest breeding values for fitness and are less prone to deleterious mutations (Hansen and Price, 1995; Beck and Powell, 2000). The difference in conclusions of these studies is a probable result of their contrary approaches towards the change in physiological state of males with age and the existence of life history trade-offs.

Therefore, in the present study, *D. ananassae* which belongs to the *melanogaster* sub group of *ananassae* complex has been used (Bock and Wheeler, 1972). In this species males do not provide parental care or nuptial gift to mated females. He can provide only sperm and accessory gland proteins to the mated female. In this species female prefers to mate with old age males more frequently than young or middle age males. Further in this species, strains both with inversion and without inversion are available. Therefore, the present study has been undertaken to understand male age influence on preadult fitness to test the hypothesis good gene model (Kokko, 1997).

Materials and Methods

Monomorphic (inversion free) and polymorphic (with inversion) strains of *D. ananassae* were established from already established (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 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 were done under Leitz Ortholux II scientific and clinical microscope both at low (10×) and high (45×) magnification. If an 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 hrs of their eclosion separately from monomorphic and polymorphic strains, were aged as required, and were cultured using same laboratory conditions 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 males into a vial containing wheat cream agar medium once in 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 show 60±2 for monomorphic and 63±2 for polymorphic strains. Since mean longevity of *D. ananassae* ranges from 60-65 days, we assigned days for young, middle, and old aged male 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 females from the respective stocks to be used in the present experiment.

Male age influence on pre adult fitness (*egg - larval hatchability and larva - adult viability*)

Unmated young, middle, old aged males and 5-6 days old virgin female were used to study male age influence on male mating activities. A female along with a male of different age classes were aspirated individually into an Elens-Wattiaux mating chamber and observed for 1 hr. Pairs unmated within 1 hr were discarded. Soon after, the mated pairs were individually transferred to a new vial once in 24 hr until their death. Hundred eggs were taken randomly using a spatula to study percentage of egg-larval hatchability and were seeded separately for each of the three male age classes in a small petridish 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 percentage of larva-adult viability, 100 1st instar larvae were collected and were placed in a petri dish containing wheat cream agar medium, and the number of adult flies emerged from these larvae were also counted, to account for percentage of larva-adult viability.

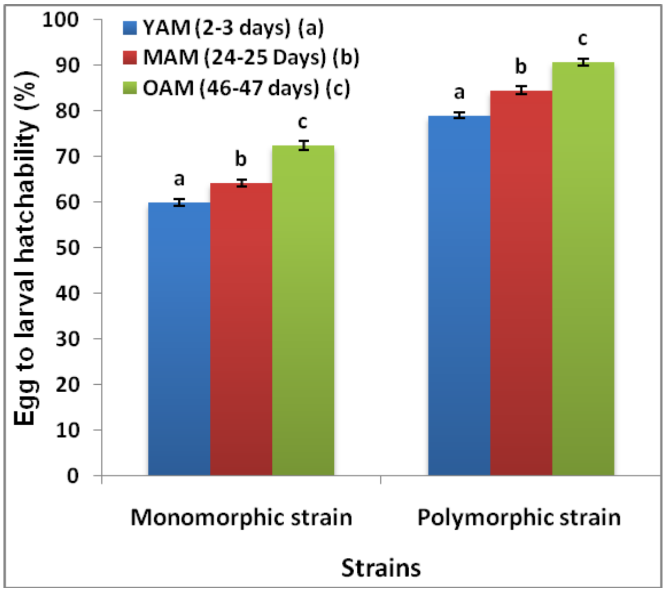

Figure 1. Male age influence on egg to larval hatchability in monomorphic and polymorphic strains of *D. ananassae*. (YAM – Young age male; MAM – Middle age male; OAM – Old age male).

Table 1. Two way Anova of male age influence on egg to larval hatchability in monomorphic and polymorphic strains of *D. ananassae.*

% Hatchability	Source	Type III Sum of Squares	df	Mean Square	F-Values
Egg to larval Hatchability	Strains	2815.203	1	2815.203	54.205**
	Male age	37380.727	2	18690.363	359.873**
	Strains*Male age	119.887	2	59.943	1.154^{NS}
	Error	15269.180	294	51.936	
	Total	1632315.997	300		

^{NS} Non-significant, **Significant at 0.0001 level.

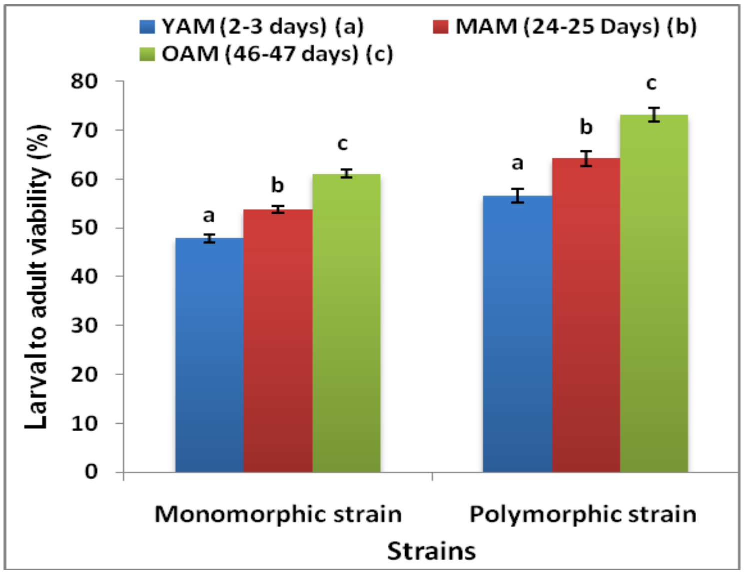

Figure 2. Male age influence on larval to adult viability in monomorphic and polymorphic strains of *D. ananassae.* (YAM – Young age male; MAM – Middle age male; OAM – Old age male).

Table 2. Two way Anova of male age influence on larval to adult viability in monomorphic and polymorphic strains of *D. ananassae.*

% Viability	Source	Type III Sum of Squares	df	Mean Square	F-Values
Larval to adult viability	Strains	4696.563	1	4696.563	84.822**
	Male age	36584.607	2	18427.303	332.804**
	Strains*Male age	720.927	2	360.463	6.510*
	Error	16278.740	294	55.370	
	Total	1312179.000	300		

*Significant at 0.005, **Significant at 0.0001 level.

Results and Discussion

Egg to larval hatchability and larva to adult viability are two important components of fitness in *Drosophila*, and they can be related to phenomena such as larval facilitation, as well as concepts of microniches and microenvironments (Castro and Mensua, 1985). Hence in the present study, these two parameters have been undertaken to study male age influence on preadult fitness in monomorphic and polymorphic strains of *D. ananassae*. Percentage of egg to larval hatchability and larva to adult viability data are provided in Figures 1 and 2, and in Tables 1 and 2. It was noticed from the table that in both monomorphic and polymorphic strains, mean preadult fitness of old aged male was found to be greater compared to preadult fitness of middle and young age males. Two way ANOVA followed by Tukey's post hock test showed significant variation in preadult fitness among males of different age classes, between monomorphic and polymorphic strains, and also in the interaction between male age classes and strains.

From the above data it was noticed that in *D. ananassae*, male age has significant influence on preadult fitness. Our study supports the theoretical models of good gene hypothesis (Kokko, 1997, 1998), suggesting that females of the species discriminate the male on the basis of male age and prefer to mate with old age males to obtain indirect genetic benefits. In the present study it was also noticed that polymorphic strains had significantly greater preadult fitness than monomorphc strain. This suggests the influence of inversion on fitness characters. This agrees with earlier studies of inversion role on fitness in different species of *Drosophila* (Spiess and Langer, 1961; Singh and Chatterjee, 1986; Singh, 1989; Sisodia. and Singh, 2001). Thus, in the present study it was clear that females of *Drosophila ananassae* preferred old age males to obtain better fitness.

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Female age influence on mating activities in outbred populations of *Drosophila ananassae*.

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Traditional models of sexual selection predict that in most animal species, males will be less discriminating in their choice of mating partners than females, because their investment in offspring is much lower (Bateman, 1948; Trivers, 1972). Costs of reproduction have been extensively studied in females, arising through offspring production but also male harassment, insemination, and maternal care (Chapman *et al.*, 1998; Roff, 2002; Harshman and Zera, 2007). However, it is becoming increasingly apparent that in many species males, nevertheless, have a high cost of reproduction (mating) due to costs arising from factors such as energetically expensive courtship displays (Judge and Brooks, 2001) and the production of ejaculates (Dewsbury, 1982; Galvani and Johnstone, 1998).

If all females in a male's pool of potential mates have equal reproductive potential, males should not preferentially mate with one over another, as maximizing only the number of female mates would give males the highest reproductive pay off (Bateman 1948). If females differ in their reproductive potential, males might exercise some degree of mate choice. Therefore, males must operate under time constraints, as well as possibly dwindling energy or sperm reserves, or both. Males that exercise mate selectivity might, therefore, have a reproductive advantage by wisely allocating their time, sperm, and energy. This is possible with females (Andersson, 1994) that provide them with the greatest gain in reproductive success.

In most of the studies on age effects of *Drosophila*, parental age on progeny fitness has not been directly looked into, but instead have considered physiological changes associated with changes in parental age, molecular aspects, selection experiments, and comparison of populations that have been generated from individuals of different ages (Parsons, 1964; Wattiaux, 1968; Ganetzky and Flanagan, 1978; Luckinbill *et al.*, 1984; Partridge and Fowler, 1992; Chippindale *et al.*, 1994; Hansen and Price, 1995). In most of these studies it was difficult to separate male age effect from female age effect. Therefore, the present study of female age effect on mating behavior has been undertaken in *D. ananassae*, a cosmopolitan domestic species of *Drosophila* belonging to *melanogaster* group of *ananassae* subgroup.

Material and Method

In the present study an experimental stock of *D. ananassae* has been established by mixing together progenies of each of the 150 naturally inseminated isofemale lines collected at domestic