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Greater fitness 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 population collected at Mysore have been used to study female age influence on fecundity, fertility, and ovariole number. It was noticed that polymorphic strains had greater fecundity, fertility, and ovariole number than monomorphic strains. It was also noticed that in both monomorphic and polymorphic strains middle aged females had significantly greater fecundity, fertility, ovariole number than young or old aged females. Thus, in *D. ananassae* middle aged females had higher reproductive fitness than young or old aged females, which is independent of inversion system. Key Words: *Drosophila ananassae*, female age, male mate preference, ovariole number, monomorphic and polymorphic.

Introduction

Male mate choice is expected in systems where males allocate valuable resources to parental investment, in response to variation in female quality or where the costs of mate search and/or assessment are

low (Bonduriansky, 2001). Further, assuming the costs of male mate choice are not too great (Altmann, 1997), it would benefit males to exercise mate choice: 1) if male reproductive success is limited by more factors than simply the number of female mates and 2) if females differ in quality or, more specifically, in their reproductive potential (Byrne and Rice, 2006). Both of the preceding criteria need to be in place so that males can mate selectively to be evolutionarily advantageous. 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 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 allocating their time, sperm, and energy wisely. This is possible with the females (Andersson, 1994) that will provide them with the greatest gain in reproductive success.

Male mating preferences have now been documented in insects (Kvarnemo and Simmons, 1999; Bonduriansky, 2001), fish (Berglund and Rosenqvist 2003; Kvarnemo *et al.*, 2007), lizards (LeBas and Marshall, 2000; Orrell and Jenssen, 2002), birds (Torres and Velando, 2005; Kraaijeveld *et al.*, 2007), and mammals (Domb and Pagel, 2001; Parga, 2006).

Male mating preferences may often serve to increase their reproductive success, as the characteristics of females that they prefer are often correlated with individual differences in fecundity or with the proximity of females to conception (Jones *et al.*, 2001; Ruscio and Adkins-Regan, 2003). Few studies have yet investigated the additional possibility that male mate choice may also affect the quality of offspring, but a recent study of house mice, *Mus domesticus*, suggests that this may be the case: when males were experimentally mated with females they did not prefer, they produced offspring with lower viability and poorer performance than those allowed to mate with preferred females (Drickamer *et al.*, 2003; Gowaty *et al.*, 2003).

The characters used by the males to select the females are generally virginity, her size, age, and gravid status (Bonduriansky, 2001). The most obvious character influencing the reproductive value of the female is her fecundity (Bonduriansky, 2001). When mating opportunities are constrained, males that show a preference for more fecund females will benefit directly by increasing the number of offspring they produce (Katvala and Kaitala, 2001). Most compelling studies of male choice suggest that female mating success is often associated with traits that are correlated with female fecundity (Bonduriansky, 2001; Byrne and Rice, 2006), while in others mate assessment may occur through display traits such as colorations, pheromones, or ornament and morphological features (Amundsen, 2000b; LeBas *et al.*, 2003). However, models of the evolution of male choice suggest that male choice will tend to break down when males target arbitrary female traits rather than those that reliably signal fecundity (Kokko and Johnstone, 2002; Chenoweth *et al.*, 2006). Therefore, male preference for female traits may be an indirect way of assessing female fecundity. Thus, there is a need to understand how male choice is related to such traits.

Therefore, the present study of maternal age influence on offspring fitness has been undertaken in *D. ananassae*, a cosmopolitan domestic species of *Drosophila* belonging to *melanogaster* group of *ananassae* subgroup.

For this purpose *D. ananassae* has been selected as the experimental model because of its following characteristics. It is a cosmopolitan domestic species belonging to *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 genetic behavior (Singh, 1985a, b). Presence of male crossing over, high level of inversion polymorphism and high mutability are the features which make it useful for certain genetic studies. Further in this species female age influence on mating success and offspring fitness has not been studied. Therefore, the present investigation was undertaken in *D. ananassae* to address the following questions: 1. Are males of *D. ananassae* able to discriminate females on the basis of female age? If so, what is its effect on female fitness and offspring quality? 2. Whether or not successful mothers produce successful offspring? 3. Whether or not inversion system has influence on inheritance of characters from female to their offspring in relation to female age?

Materials and Methods

Experimental stocks of monomorphic and polymorphic strains of *D. ananassae* were established from isofemale lines of *D. ananassae* obtained from out-bred populations collected at semi domestic localities in Mysore, Karnataka, India (Prathibha and Krishna, 2010). These isofemale lines were maintained at $21\pm 1^\circ\text{C}$ and relative humidity of 70%. When larvae appeared, eight third instar larvae from each isofemale line were used to analyze presence or absence of inversions. This procedure was continued for 3 generations to establish monomorphic (inversion free) and polymorphic (with inversion) strains and to allow flies to acclimatize in the laboratory. In the 4th generation 5-6 days old flies were used to collect synchronized eggs (± 30 min) using Delcour's procedure (1969). Eggs (100) were seeded in a vial containing wheat cream agar medium. When adults emerged, virgin females and unmated males were isolated within 3 hr of their eclosion and were aspirated into a new vial containing wheat cream agar medium. These flies were aged as required for the experiment: young aged females (2-3 day old), middle aged females (17-18 days old), and old aged females (32-33 days). These females were kept individually in a culture vials containing wheat cream agar medium and were transferred to a new vial containing wheat cream agar medium once a week until they were used in the experiment. Male flies were aged in the same environment for 5-6 days.

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

To study relation between female age related changes in fecundity and fertility and inversion system, a male (5-6 days old) along with a female (young/ middle/ old) were aspirated into an Elens-Wattiaux mating chamber (1964) and observation was made for 1 hr. Soon after mating, the mated female was transferred individually into a fresh vial containing wheat cream agar media once in 24 hr to study fecundity and fertility, and this was continued for 16 days. Total number of eggs laid by each pair and total number of flies emerged from each pair was recorded. A total of 50 trials were made separately for each of the three female age classes. Separate experiments were carried out for monomorphic and polymorphic strains. Two Way ANOVA followed by Tukey's Honest Post hoc test was carried out on fecundity and fertility data using SPSS 10.1 software.

Female age influence on ovariole number in monomorphic and polymorphic strains

Virgin young/middle/old aged females were individually sacrificed to count number of ovarioles following the procedure of Krishna and Hegde (1997). To count ovariole number, each female was dissected in a drop of physiological saline using binocular stereomicroscope; ovarioles were separated from one another from the left ovary with the help of fine needles. Number of ovarioles in each female was counted. Mean ovariole number data were also subjected to two way ANOVA followed by Tukey's Honest Post hoc test. Separate experiments were carried out for monomorphic and polymorphic strains.

Results

Mean fecundity and fertility of young, middle aged, and old females mated to males in monomorphic and polymorphic strains are provided in Figure 1a and Table 1b. Two way ANOVA followed by Tukey's Honest Post hoc test (Tukey's test) carried out on fecundity and fertility data showed significant variation between strains and between female age classes. However, it shows insignificant difference in the interaction between strains and female age classes. Tukey's test showed middle aged females had significantly greater fecundity and fertility compared to young or old aged female. Young aged females had greater fecundity and fertility than old aged females.

Polymorphic strains had greater mean number of ovarioles compared to monomorphic strains. In both monomorphic and polymorphic strains mean number of ovarioles was found to be highest in middle aged female while lowest in old aged females (Figure 2a and Table 2b). Mean ovarioles data of monomorphic and polymorphic strains subjected to two way ANOVA followed by tukey's test showed significant variation between female age classes (young, middle, and old) between strains (monomorphic and polymorphic) and also interaction between female age and strains. Tukey's test showed that middle aged females had significantly greater ovariole number compared to young or old aged females. Similarly young aged females

had significantly greater ovariole number than old aged females. Polymorphic strains had significantly greater ovariole number compared to monomorphic strains by Tukey's test.

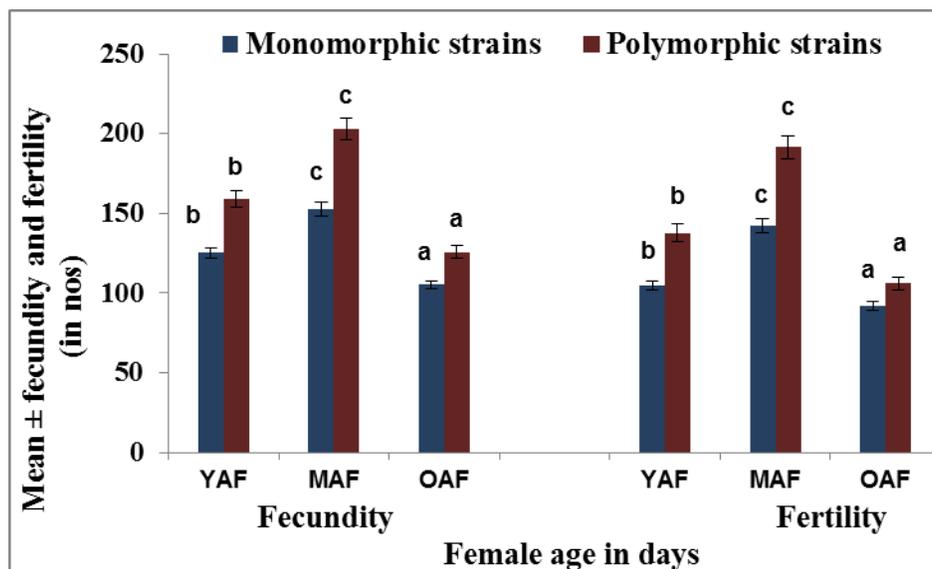


Figure 1a. Female age influence on female fecundity and fertility in monomorphic and polymorphic strains of *D. ananassae* (Values are mean \pm SE). **YAF**- Young age female; **OAF**- Old age female; **MAF**- Middle age female. Different letter on bar graph indicates significant by Tukey's test.

Table 1b. Female age influence on female fecundity and fertility in monomorphic and polymorphic strains of *D. ananassae*.

Parameters	Source	Type III Sum of Squares	df	Mean Square	F-Values
Fecundity (in no)	Strains	90688.853	1	90688.853	85.001**
	Male age	196104.807	2	98052.403	91.903**
	Strains \times Male age	10778.447	2	5389.223	5.051*
	Error	313671.280	294	1066.909	
	Total	6926866.000	300		
Fertility (in no)	Strains	77666.430	1	77666.430	73.746**
	Male age	240433.047	2	120216.523	114.148**
	Strains*Male age	15115.220	2	7558.110	7.177*
	Error	309629.220	294	1053.161	
	Total	5636435.000	300		

* Significant at 0.05 level, **Significant at 0.0001 level.

Discussion

Figure 1a and Table 1b show that in both monomorphic and polymorphic strains middle aged females had significantly greater fecundity and fertility over young or old aged females suggesting that females of the same species show variation in reproductive potential across the age. This confirms the earlier works suggesting that female age is also one of the traits known to influence female fecundity (Bonduriansky, 2001; Jones, *et al.*, 2001; Schamel *et al.*, 2004; Marinkovic, 1967; Singh and Chaterjee, 1987, 1988; Katvala and Kaitala, 2001; Long *et al.*, 1982); working on the evolution of male mate choice in insects has also found influence of female age on female fecundity.

Since ovariole number is positively correlated with fecundity, we sacrificed females of different age classes to study ovariole number variation, if any, across the female ages. Figure 2a and Table 2b show that middle aged females had significantly greater ovariole number over young or old aged females in monomorphic and polymorphic strains and followed the similar pattern of fecundity variation across the

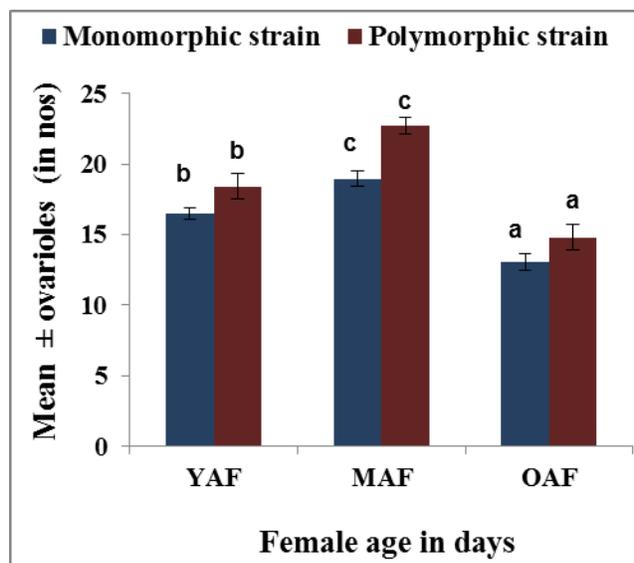


Figure 2a. Female age influence on female ovariole number in monomorphic and polymorphic strains of *D. ananassae*. YAF- Young age female; OAF- Old age female; MAF- Middle age female.

Table 2b. Female age influence on female ovarioles number in monomorphic and polymorphic strains of *D. ananassae*.

Ovarioles	Source	Type III Sum of Squares	df	Mean Square	F-Values
Ovarioles (in no)	Strains	461.280	1	461.280	68.045**
	Male age	2394.647	2	1197.323	176.621**
	Strains×Male age	63.780	2	31.890	4.704*
	Error	1993.040	294	6.779	
	Total	95880.000	300		

* Significant at 0.05 level, **Significant at 0.0001 level.

female ages. This again confirms the earlier studies of female age influence on reproductive success in other insects, too (Bonduriansky, 2001; Branquart and Hemptinne, 2000; Partridge *et al.*, 1987). From the results it was also noticed that in *D. ananassae* middle aged females had significantly greater ovariole number, fecundity, and fertility compared to young or old aged females in both the strains studied. Therefore, it is advantageous for the male to mate with middle aged females compared to young or old aged females. However, it is not known why older females had less ovarioles compared to young or middle aged females? Whether they reabsorbed as female age, we do not have answer for this.

Thus, these studies suggest that males of *D. ananassae* exercised mate choice to obtain direct fitness benefits, and we also found occurrence of age specific reproductive success in females of *D. ananassae* which is independent from influence of inversion system.

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Newly recorded inversion and re-annotation of inversion breakpoints in *Drosophila cardini* species.

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Abstract

This report presents a new paracentric inversion in the middle of the chromosome X in *Drosophila neocardini*. Also, a careful analysis was carried out of chromosomal inversion breakpoints that were previously described for *Drosophila polymorpha*. Photo comparison, and the release of a newly designed photomap, allowed us to introduce changes to the breakpoints of In(2R)A and In(2R)D. All individuals analyzed were collected in two conserved areas in Santa Catarina/south of Brazil. Key words: chromosomal polymorphism, cytogenetic, polytene chromosomes.

Introduction

Chromosomal inversions were first discovered by Sturtevant in 1917 as the recombination modifier in *Drosophila melanogaster*, that occurs when a chromosome breaks at two points and the segment bounded by the breakpoints is reinserted in the reversed orientation (Navarro *et al.*, 2000). This inversion mutation that reordered genes in the genome became a powerful genetic marker to assess naturally occurring genetic variation (Wallace *et al.*, 2013). Also, the ability to identify inversions with simple cytological analysis proved to be very important in these early studies (Painter, 1933).

The cytological analysis involves a technique with polytene chromosomes present in the nuclei of salivary glands of *Drosophila* species. These chromosomes allow easy identification of the arrangements in heterozygotes, since they in this case result in an inversion loop. Different types of inversion are classified into two basic categories: pericentric inversions that include a centromere region, and paracentric inversions, that do not. Paracentric inversions are also the more common type of chromosomal inversion (Krimbas and Powell, 1992).