(MBENs) and lateral horn (LH) neurons of the brain of both the age group flies, the activity of ChAT was not affected by training (Figure 1).

In the case of young flies the activity of ChE enzymes was significantly increased in trained flies, but the extent of enhancement was considerably higher in AchE (Figure 2). Surprisingly, 50-day-old trained flies exhibit decreased enzyme activity compared to untrained ones. AChE shows more reduction compared to BChE (Figure 2).

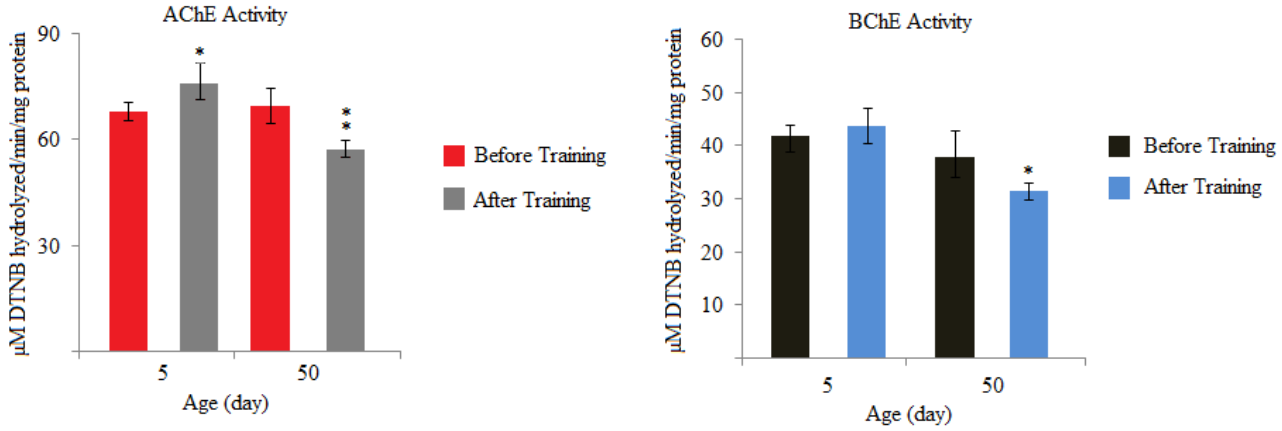

Figure 2. Activity of ChE enzymes.

Age-related decrease in the activity of ChAT accompanied with constant activity of ChE enzymes were observed in older flies. Lack of enhancement in the activity of ChAT and noticeable decrease in ChE enzymes at the time of training in aged flies can induce a process, which could lead to lower levels of acetylcholine, compromising the neuronal plasticity and impaired memory in the aging brain.

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The effect of pyrogallol on the pre-adult fitness of *Drosophila bipectinata.*

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Abstract

The effect of pyrogallol (an antioxidant) on the pre-adult fitness has been studied using an outbred population of *D. bipectinata*. It was noticed that there was a decrease in the egg to larva, larval to pupal, and pupal to adult development and the rate of development in the pyrogallol treated *D. bipectinata* flies, which had significantly lower pre-adult viability and rate of development, when compared to the untreated flies. The SOD levels were significantly greater in untreated flies than the

treated flies. Among the treated flies, SOD levels decreased with an increase in the concentration of pyrogallol in the diet. Thus, these studies suggest that, in *D. bipectinata*, pyrogallol (an antioxidant) has a negative effect on the product viability and the rate of development of the fly. Key Words: Antioxidants, Superoxide Dismutase, Viability, Rate of development

Introduction

The net fitness of an organism is determined by various components which can be divided into two major categories: the pre-adult fitness and the adult fitness (John *et al.*, 1981). Every character may contribute to the total fitness of the organism through one or more of the many biochemical and physiological pathways in the developmental process. An interrelation of these pathways can be deduced, which is complicated but can be categorized distinctly (Yuichiro *et al.*, 1960). The rate of development, along with pre-adult fitness is known to be highly correlated, while the rate of development (time from egg laying to eclosion) is considered as a major component of the pre-adult fitness of an organism. The pre-adult viability is measured by the survival through the pre-adult stages. Therefore, a study of developmental rate also gives information about viability (Bonnier *et al.*, 1959).

Many studies have been carried out in *Drosophila* at the intraspecific level, using quantitative traits to investigate climatic adaptations by comparing flies' performance in laboratory assays, *e.g.*, development time (James *et al.*, 1995; Norry *et al.*,2001; Sgro and Blows, 2003; Griffiths *et al.*, 2005), larval growth efficiency (Robinson and Partridge., 2001), larval survival and pre-adult competitive ability (James and Partridge, 1998), stress resistance (Krebs and Loeschcke, 1995; Karan *et al.*, 1998a; Bubliy *et al.*, 2002; Hoffmann *et al.*, 2002, 2005). Also, the effects of light regimes, temperature along with the region of occurrence (like temperate and tropical areas), and male age on the pre-adult fitness have been studied.

Parental environment and fitness have also been correlated with that of the offspring, thus indicating that an enhancement in the parental environment will lead to enhanced offspring phenotype (Fox *et al.*, 1997; Donohue and Schmitt., 1999). Nutritional enhancements in the diet are known to enhance fitness (Djawdan *et al.*, 1998). Work by Anderson *et al*. (2010), shows that larval nutrition affects a wide range of life history traits, tested by assaying the heat, cold and desiccation tolerance in the larvae raised on carbohydrate and protein enriched medium. They also showed that the increased egg to adult viability in media rich in sucrose along with the positive effects of protein enrichment on the female survival. Thus, indicating that nutrition at the pre-adult stages plays a major role in the overall fitness of the adult. The different levels of food available at these stages also cause the regulation of the resource processing. In addition to this they showed that lower levels of food leads to lower body size (Zoltan and Gerdien, 2003), which is a general phenomenon in ectotherms (Atkinson and Sibly, 1997) and is shown in *Drosophila melanogaster* and other species by Gebhardt and Stearns (1998).

The complete nutrition of an organism in nature consists of carbohydrates, proteins, lipids, fats, and antioxidants at different concentrations. Although many experiments have been done using carbohydrate and protein enrichment in media, antioxidant enrichment and its effects have seldom been carried out. Thus in this experiment the effect of enhanced quantities of antioxidants in the diet are measured on the pre-adult fitness and the rate of development tested in the species *D. bipectinata*, which is a complex of the *ananassae* subgroup of the *melanogaster* species group, a member of the *bipectinata* species (Bock and Wheeler, 1972).

Materials and Methods

Establishment of stock:

The experimental stock of *Drosophila bipectinata* was established from progenies of 50 iso-female lines collected from Chamundi Hills at Mysore, Karnataka, using mixed fruit bait. These flies were cultured in a standard wheat cream agar medium and maintained at a constant temperature of 22 ± 1ºC with a relative humidity of 70%. In each generation, the emerged flies were mixed together and redistributed to ten new culture bottles each with 20 flies (10 males + 10 females). This procedure was continued for 3 generations to acclimatize the flies to laboratory conditions. At the fourth generation eggs were collected using Delcour's procedure (1969) and 100 eggs were seeded in new culture bottles. When adults started emerging, virgin females and males were isolated within 3 hours of their eclosion. These flies were aged for 5-6 days to be used in the present experiments.

Preparation of antioxidant media at different concentrations:

The antioxidant pyrogallol was dissolved in water at a concentration of 1000 ppm (*i.e*., 1 g in 100 ml of distilled water), which was maintained as stock solution, from which different amounts were taken and mixed in 30 ml of the standard wheat cream agar media in concentrations of 1 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm, respectively, to check the LD_{50} concentration of the compound (that is, the concentration at which 50% of the progeny dies). The LD_{50} was found to be 10 ppm. The flies raised in three sublethal doses of 1 ppm, 3 ppm, and 5 ppm were used for the present experiments.

Effect of pyrogallol on SOD enzyme levels:

Enzyme preparation for Superoxide Dismutase analysis: The flies cultured on different sub-lethal concentrations of 1 ppm, 3 ppm, and 5 ppm of pyrogallol were subjected to assay the effect of pyrogallol on the stress enzyme Superoxide Dismutase (SOD). To assay this, five adult (5 males and 5 females) flies from each concentration were taken along with the untreated (control) in different Eppendorff tubes. 200 microlitres of 50 mM Phosphate buffer of pH 7 for Catalase assay, 200 microlitres of 250 mM Phosphate buffer of pH 7.8 for SOD Assay were added and crushed using tissue homogenizer in ice cold conditions and centrifuged at 8000 rpm for 20 min in a cooling Microfuge. After this, the supernatants were poured into other eppendorff tubes. 0.1 ml/100 µl of this enzyme extract was used for the assay.

Measurement of Superoxide Dismutase enzyme activity: SOD enzyme was assayed using a modified procedure originally described by Beauchamp and Fridovich (1971). The enzyme extract was added to a mixture of solutions containing 250 mM phosphate buffer (0.8 ml), 100 mM Methionine (1 ml), 100 mM Riboflavin (0.5 ml), 5mM EDTA (0.1 ml), 750 mM NBT (0.1 ml), the volume of which was made up to 3 ml with distilled water. A mixture without the enzyme and NBT was prepared which served as a blank, and a control with NBT and no enzyme was prepared. These mixtures are exposed to sunlight or a bright light of about 400 watts; during this reaction NBT gets reduced to formazone, and was read at 560 nm. The total protein content of the enzyme was estimated by Lowry's method and the activity was expressed in units /mg of protein.

Effect of pyrogallol on Pre adult fitness parameters:

(a) *Egg to larval hatchability*:

Eggs were collected separately from the cultures of untreated and pyrogallol treated flies (1 ppm, 3 ppm, 5 ppm). Eggs were collected by Delcor's procedure (1969) to study the effect of

pyrogallol on the egg to larval hatchabilty. Eggs (100) were seeded separately into each vial (1 × 3 cm) containing ~ 6 ml of wheat cream agar media (control) and pyrogallol at different sublethal concentrations. The total number of eggs that hatched into larvae was counted using a stereomicroscope at 40× magnification. A total of 10 replicates was run separately for both untreated and pyrogallol treated (1 ppm, 3 ppm, 5 ppm) flies.

(b) Larval to pupal viability:

First instar larvae were collected separately from the cultures of normal and pyrogallol treated flies (1 ppm, 3 ppm, 5 ppm) were used to study the effect of pyrogallol on larval to pupal viability. First instar larvae (100) were seeded separately into each vial (1 × 3 cm) containing ~ 6 ml of wheat cream agar media (control) and pyrogallol at different sublethal concentrations. The total number of pupae formed from these larvae was counted. A total of 10 replicates was run for both untreated and pyrogallol treated (1 ppm, 3 ppm, 5 ppm) flies.

(c) Pupal to adult eclosion:

To study the effect of pyrogallol on the pupa to adult eclosion, pupae were obtained from both untreated and pyrogallol treated cultures (1 ppm, 3 ppm, 5 ppm). Pupae (100) were transferred separately into each vial (1 × 3 cm) containing ~ 6 ml of wheat cream agar media (control) and pyrogallol at different sublethal concentrations. The total number of adults eclosed from the pupae was counted. A total of 10 replicates was run for both untreated and treated (1 ppm, 3 ppm, 5 ppm) flies.

The data obtained from each of the above experiments (egg to larval hachability, larval to pupal viability, pupal to adult eclosion), was subjected to a one-way ANOVA analysis followed by the Tukey's *Post Hoc* test.

Table 1. Effects of pyrogallol on the pre-adult viability of *D.bipectinata.*

Pre-adult viability	Untreated (Control)	Pyrogallol treated flies			F-value
		1 ppm	3 ppm	5 ppm	
Egg to larval hatchability	65.1 ± 4.37^{a}	40.5 ± 1.87^{b}	49.7 ± 2.44^{b}	48.9 ± 2.15^{b}	26.72*
Larval to pupal viabilty	55.1 ± 4.12^{a}	26.1 ± 2.25^{b}	16.1 ± 0.9^{c}	14.6 ± 0.91^{c}	713.39**
Pupal to adult eclosion	39.2 ± 3.32^{a}	11.8 ± 1.13^{b}	8.1 ± 0.62^{b}	8.5 ± 0.92^{b}	141.551***

*Significant at p<0.01; **Significant at p<0.0001; ***Significant at p<0.001;
Different letters in the super script indicate significance at 0.05 levels by Tukey's *post hoc* test.

Effect of pyrogallol on the rate of development of pre-adult stages:

Eggs were collected separately from flies of both untreated and pyrogallol treated (1 ppm, 3 ppm, 5 ppm) media using Delcor's procedure (1969). One hundred eggs/larvae/pupae were transferred separately to vials containing ~ 6 ml of normal and pyrogallol treated (1 ppm, 3 ppm, 5 ppm) media. The number of hours taken by each egg to hatch into larvae was observed. Similarly, the number of days taken by the first instar larva to form pupa and the pupa to eclose to adult were also observed and recorded. One-way ANOVA analysis followed by the Tukey's *post hoc* test was carried out on the data on the rate of development of the pre-adult stages.

Results

Effect of pyrogallol on Superoxide Dismutase enzyme activity:

The Figure 1 shows the SOD enzyme levels in the pyrogallol treated males of *D. bipectinata.* It was observed that the SOD enzyme levels were found to be higher in the untreated males when compared to the treated males. Among the pyrogallol treated males the levels of SOD were found to have decreased with an increase in the concentration of pyrogallol. The lowest level of the SOD enzyme was found at the 5 ppm concentration and the highest was at the 1 ppm concentration. This suggests that in males of *D. bipectinata* SOD activity decreases with increasing pyrogallol concentration.

SOD enzyme levels of untreated and pyrogallol treated females of *D. bipectinata* is depicted in Figure 2. SOD levels were found to be higher in the untreated when compared to the pyrogallol treated females. Among the pyrogallol treated females, SOD activity was the least at 5 ppm and highest at 1 ppm. Further, SOD activity decreased with an increase in the concentration of pyrogallol. Between the sexes the SOD enzyme levels in the female flies were lower than that of the males, both in the untreated and the pyrogallol treated groups, suggesting that the females had a lower level of SOD enzyme activity than the males.

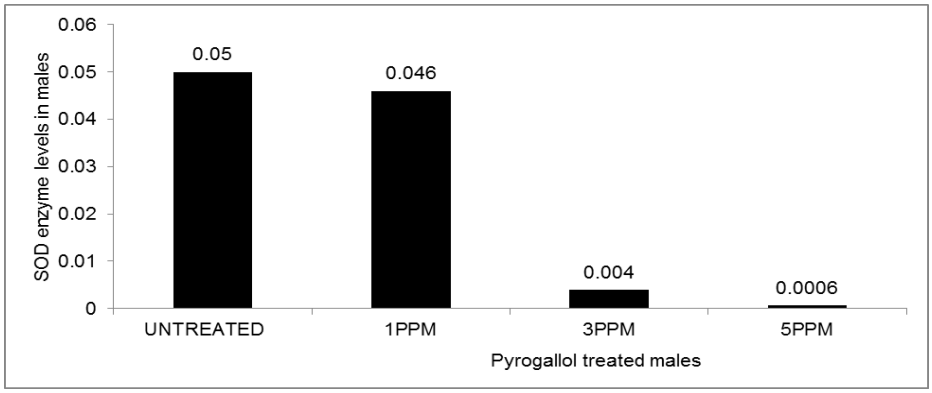

Figure 1. Effects of pyrogallol on the SOD levels in males of *D. bipectinata.*

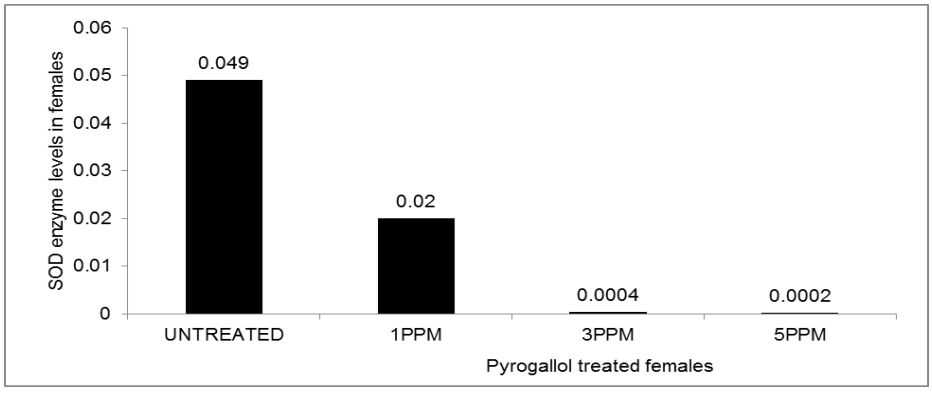

Figure 2. Effects of pyrogallol on the SOD levels in females of *D. bipectinata.*

Effect of pyrogallol on Pre adult fitness parameters:

Table 2 shows the mean values of egg to larval hatchability, larval to pupal viability, and pupal to adult eclosion in untreated and pyrogallol treated groups. The number of eggs that hatched into larvae, larvae that formed pupae, and pupae that eclosed into adults was higher in the untreated when compared to the pyrogallol treated groups. Among the pyrogallol treated group the egg to

larval hatchability also varied, with the least egg to larval hatchability noticed in the sequence of 1 ppm < 5 ppm < 3 ppm, while the larval to pupal viability showed variation with the least viability in the 5 ppm concentration and the highest in 1 ppm. The pupal to adult eclosion showed a decrease in the eclosion with increased concentration of pyrogallol. Flies rared at 1 ppm concentration of pyrogallol had the highest pupal to adult eclosion and the least pupal to adult eclosion was noticed at the 5 ppm concentration.

One-way ANOVA followed by the Tukey's *post hoc* test carried out on the egg to larval hatchability, larval to pupal viability, and pupal to adult eclosion data obtained, showed significant variations between untreated and pyrogallol treated groups. From the Tukey's *post hoc* test it was found that there was a significant difference in the egg to larval hatchability between the untreated and pyrogallol treated groups. And among the pyrogallol treated group, egg to larval hatchability was found insignificant by the Tukey's *post hoc* test. While the larval to pupal viability in 1 ppm was found to significantly lower than the untreated flies, it was significantly higher when compared to 3 ppm and 5 ppm pyrogallol concentration. However, larval to pupal viability was found to be insignificant between 3 ppm and 5 ppm pyrogallol concentrations, and the pupal to adult eclosion showed a significant difference in the eclosion between the untreated and treated groups; among the treated there was no significant variation between 1 ppm and 3 ppm and 5 ppm concentrations.

Table 2. Effects of pyrogallol on the rate of development in *D.bipectinata.*

Rate of development (in hours)	Untreated (Control)	Pyrogallol treated flies			F-value
		1 ppm	3 ppm	5 ppm	
Egg to larava	8.64 ± 0.008^{a}	12.05 ± 0.008^{b}	13.25 ± 0.11^{c}	15.54 ± 0.21^{d}	479.45*
Larval to pupa	60.64 ± 0.26^{a}	72.00 ± 0.31^{b}	95.98 ± 0.19^{c}	120.98 ± 0.34^{d}	8858.63*
Pupal to adult	92.61 ± 0.25^{a}	120.00 ± 0.33^{b}	144.30 ± 0.27^{c}	96.01 ± 0.21^{d}	7929.28*

* Significant at $p < 0.001$;

Different letters in the super script indicate significance at 0.05 levels by Tukey's post hoc test.

Effect of pyrogallol on the rate of development of pre-adult stages:

Table 2 shows the mean values of rate of development from egg to larva, larva to pupa, and pupa to adult. The rate of development was the lowest in the untreated when compared to the pyrogallol treated groups, and it increased with an increase in the concentration of pyrogallol among the treated groups. In contrast to this, the rate of development of the pupa among the pyrogallol treated groups was least in the 5 ppm concentration, followed by 1 ppm and 3 ppm.

The data when subjected to a one-way ANOVA followed by the Tukey's *post hoc* test showed significant variation in the rate of development in egg to larvae, larvae to pupae, and pupae to adult, between the untreated and pyrogallol treated groups. Among the treated groups, in the egg to larval and larval to the pupal rate of development, there was a significant variation between concentrations in the 5 ppm concentration having significantly higher rates of development than the 1 ppm and 3 ppm groups, with significant variations also seen between the 1 ppm and 3 ppm groups by the Tuckey's *post hoc* test. However, in the pupal to adult development there was a significant variation between concentrations in the rate of development in the 3 ppm concentration being significantly higher than the 1 ppm and 5 ppm groups, with significant variations also seen between the 1 ppm and 5 ppm groups by the Tuckey's *post hoc* test. The least developmental rate was seen in the 5 ppm concentration.

Discussion

The developmental theory of ageing suggests a positive correlation between rate of development and adult longevity (Zwaan *et al.,* 1991), although other studies by Lints and Lints (1978) and Lints (1985) have shown that there is a negative correlation between lifespan and developmental rate. Since antioxidants are known to increase longevity by decreasing the oxidative stress in an organism, the effect of the antioxidant pyrogallol was tested in order to determine this correlation. From the results obtained, in the SOD enzyme levels, there was a decrease in the SOD level with an increase in the concentration of pyrogallol, indicating that there is an evident effect of pyrogallol on the fly. Thus testing for the pre-adult fitness along with the rate of development between the untreated (control) and pyrogallol treated groups estimates the effects of antioxidants on the fly.

A variation in the egg to adult hatchability along with the mean values of the rate of development was noticed. The egg to adult hatchability decreased with the increase in concentration of pyrogallol in the media, while the rate of development was higher with an increase in pyrogallol. This comparison can be used to infer that the antioxidant pyrogallol also plays a significant role in the pre-adult fitness as well as the rate of development in the treated flies.

Temparature has been shown to affect the rate of development and the pre-adult survivability, where lower the temperature, slower the development (Santos *et al.,* 2006) and higher the chances of survival (Partridge *et al.,* 1994). Hence, under the controlled conditions of the lab the temperatures are regulated at 22 ± 1°C, to ensure temperature does not act as a trade-off between the effects of the antioxidants on the rate of development and the developmental time.

Studies have also shown that larval crowding can have an effect on the pre-adult growth and rate of development (Santos *et al*., 2006) causing a reduction in growth efficiency; however, it was ensured in our experiments that the larval number per vial was maintained to a maximum number of 20, which rules out the effect of crowding on the results obtained. The male age is another known factor to play an important role on the pre adult fitness, showing that older males are preferred by the female in order to gain better genetic benefits (Prathibha *et al*., 2012); therefore, it was ensured that the males and females were of the same age to ensure that there are no such effects. In addition to this light is known to play a role in the rate of development. Studies have shown that flies exposed to continuous light show faster development rates than the flies exposed to light and dark periods.

Nutrition is known to enhance fitness (Djawdan *et al.,* 1998), agreeing to the work by Anderson *et al.* (2010) that larval nutrition like enrichment of carbohydrate and protein affects a wide range of life history traits, along with the increased egg to adult viability in media rich in sucrose along with the positive effects of protein enrichment on the female survival, indicating that nutrition at the pre-adult stages plays a major role in the overall fitness of the adult. In our results there was a lower viabilty in the larval stages after antioxidant enrichment, indicating the effect of the antioxidant pyrogallol, which was administered through the diet.

Even though antioxidants are known to increase the longevity of flies, studies have shown that there is no correlation of the pre-adult fitness and the longevity a fly (Zwaan *et al.,* 1991). Hence the results obtained certify that the effect of pyrogallol was the sole reason for the results obtained as all the other factors that may have played a role were kept constant. Thus, pyrogallol has a role to play in the rate of development and the survivability of pre adult stages in *D. bipectinata*.

Acknowledgments: We thank the chairman, Department of Studies in Zoology for the facilities.

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Male age influence on mating activities of monomorphic and polymorphic strains of *Drosophila ananassae.*

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Introduction

Females of a species select males to derive either direct or indirect fitness benefits. Therefore, male success in mating depends on his ability to provide material benefits to mating female or genetic benefit to their offspring. Thus, females of a species use a variety of male phenotypes to select the potential mate (Anderson, 1994). Male age is one such trait used as reliable signal to determine male quality as has been shown in earlier studies of age based female mate choice experiments (Avent *et al.*, 2008; Beck and Powell, 2000). 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 the effects of indirect benefit unequivocally requires exploration of the effects of female mating preferences on offspring survival and reproduction.

In species of *Drosophila* inversion karyotypes are known to influence on mating activity, mating advantage, mating speed, and female mate preference (Spiess and Langer, 1964a, b; Day and Butlin, 1987). It was also found that age based female mate preference is in a few species of