between the sexes. Females, the longer lived sex, showed greater resistance to ethanol-induced mortality when compared with that of males (Figure 2). Ethanol-induced differential oxidative stress correlates with that of antioxidant status in the sexes (detailed results will be published elsewhere). Our results strongly point out the marked sex difference in ethanol-induced mortality which positively correlates with the antioxidant defense mechanisms in the sexes. Our study presents evidence for the possible role of oxidative stress in the gender difference in longevity of *D. melanogaster*.

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Age based male mate preference in *Phorticella straita*.

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Introduction

Studies of sexual selection have largely concentrated on females, as they are often the selective sex that chooses from among males (Milinski, 2001). Although female-biased empirical evidence were more in the literature, males are also likely to demonstrate mate choice under some conditions (Dewsbury, 1982). Therefore, male mate choice is also expected in systems where males allocate valuable resources to their partner, in response to variations in female quality, or where the costs of mate searching and/or assessment are low (Boundsuriansky, 2001).

It was found that there are now growing numbers of observations of male choice seen in a wide range of taxa, and it has been reported in 58 insect species distributed among 11 orders and 37 families (Bonduriansky, 2001; Byrne and Rice, 2006), birds (Jones and Hunter, 1993), and fish (Amundsen and Forsgren, 2001). Studies of male mate choice in these organisms is predicted to be adaptive when variance in female fitness is large and males experience costs of mating such that they cannot inseminate all females encountered (Burley, 1977; Parker, 1983; Owens and Thompson, 1994; Johnstone *et al.,* 1996; Kokko and Monaghan, 2001). However, the empirical evidence is limited.

It was also suggested that unlike females, males also use characters in females such as virginity, body size, age, and gravid status (Bonduriansky, 2001; Prathibha and Krishna, 2010; Somashekar and Krishna, 2011). However, the most obvious character influencing the reproductive value of a female is her fecundity (Bonduriansky, 2001). If mating opportunities are constrained, of males then he shows a preference for more-fecund females to obtain direct benefit by increasing the number of offspring they produce (Katvala and Kaitala, 2001).

The 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 coloration, pheromones, or ornamental and morphological features (Amundsen, 2000; Chenoweth and Blows, 2003; Lebas *et al.*, 2003). However, models of the evolution of male choice suggest that male choice tends to break down when males target arbitrary female traits rather than those that reliably signal fecundity (Kokko and Johnstone, 2002; Chenowethet *et al.,* 2006; Servedio and Lande, 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 has been undertaken in *P. straiata* to understand male choice for female fecundity.

P. *straiata* (Nirmala and Krishnamurthy, 1975) is a Drosophillid insect discovered from Karnataka, India, belonging to group Drosophillidae. This species also has all characteristics of a good laboratory tool to analyze genetic and evolutionary problems as that of the genus *Drosophila* (Sarath Chandra and Hegde, 2003). In this species the size related mating and reproductive success has been studied by Sarath Chandra and Hegde (2003). They found that larger flies had greater reproductive success than small flies. But for this maiden lone attempt no work has been done on behavioral genetics, cytogenetics, and evolutionary genetics of this species. Also, no work so far has been done to study female age effect on reproductive performance. Therefore, the present investigation has been undertaken in *P. straiata* to test whether males of this species are able to discriminate their mates on the basis of female age classes to obtain fecundity benefit or not.

Materials and Methods

Establishment of experimental stock

The experimental stock of *P. straiata* was obtained from the progenies of 50 isofemale lines collected from Chamundi hills, Mysore, India. In each generation 20 males and 20 females were transferred to the *Drosophila* culture bottle containing wheat cream agar media. These bottles were maintained at $22\pm1^{o}C$ and at a relative humidity of 70%. From these bottles, the virgin females and males were isolated within 3 hr of eclosion and were aged as required in the present experiment.

Selection of female age class

The sexually mature *Drosophila* females will perform certain of the behaviors, such as decamping, wings flicking, leg kicking, and ovipositor extrusion on unwanted advances of courting males (Spieth, 1952). The newly emerged females will not show these rejection behaviors (Manning, 1961). Further, the immature females are characterized by unhardened cuticles, folded wings, and slow movements.

Before assigning the age classes, the reproductive activities of females were studied. The observations showed that females were unreceptive on the day of eclosion and show no courting behavior toward males. From 2-32 days, the females were receptive and showed rejection responses, such as decamping, ignoring, wings flicking, and leg kicking. After 32 days, these behaviors began to decline. Therefore 2-32 day old females were considered in the present experiment. Because the females take 15-16 days to lay eggs and remate, three age classes were created, each separated by 15 days: young aged (2-3 days); middle aged (17-18 days); old aged (32-33days). Flies of these three age classes were collected and separately maintained under uniform environmental conditions. Additionally, 5-6 day old unmated males were maintained individually in the same laboratory conditions.

Before beginning the experiment, developmental times of flies collected at different times were also tested, and the results indicated no significant variations. These females of different age classes were kept in groups of 4 flies each in 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 for 5-6 days. These flies were also maintained using the above-described laboratory conditions until they were used in the experiment.

Age based male mate preferences

To study male mate preference for female age, 2 virgin females (younger *vs*. middle-aged, younger *vs*. older, or middle-aged *vs*. older) and a 5-6 day-old male were aspirated into an Elens-Wattiaux mating chamber

(1964). Indian ink was painted on the thorax of one of the females. The effect of paint was tested before commencing the experiment by painting young flies in one trial and middle-aged/older females in an alternate trial and allowing them to mate. In 28 of 50 trials, middle aged females mated, and in the remaining 22 of 50 trials older females mated ($\chi2 = 0.72$; *d.f.* = 1; $p > 0.05$). Results showed insignificant differences suggesting that painting had no influence on the performance of the flies. Each pair was observed for 1 h. When mating occurred, the copulating pair was aspirated out of the mating chamber and placed in a new vial containing wheat cream agar medium. In total, 50 trials were conducted for each combination of female ages. A Chi-square analysis was carried out to examine male mate choice data.

Influence of female age on ovariole number and wing length
     Virgin young, middle-aged, and old females were individually sacrificed to count the number of ovarioles and determine the female wing length following the procedures of Krishna and Hegde (1997). To count the number of ovarioles, each female was dissected in a drop of physiological saline using a binocular stereomicroscope; ovarioles of the left ovary were separated from one another with the help of fine needles. The number of ovarioles in each female was counted. From the same female, wing length was also measured using a 100× [microscope] following the procedures of Hegde and Krishna (1997). The mean ovariole number and female wing length data were also subjected to one-way ANOVA followed by Tukey's honest *post-hoc* test. In total, 50 trials were separately conducted for each of the 3 female age classes.

Table 1. Age based male mate preference in *P. straita.*

Male (5-6 days)	Females (N = 50)			Females (N = 50)			Females (N = 50)		
	Young	Old	χ^2 value	Young	Middle aged	χ^2 value	Middle aged	Old	χ^2 value
Number	30	20	2*	15	35	8**	34	16	6.48**
%	60	40		30	70		68	32	

*P < 0.05 level; **P < 0.01 level;

Table 2. One way ANOVA on ovarioles number, fecundity and female wing length in *P. straita.*

Parameter	Source	Type III Sum of Squares	df	Mean Square	F value
Ovarioles (in no)	Age	4449.293333	2	2224.647	176.66**
	Error	1851.08	147	12.59238	
	Total	137606	150		
Fecundity (in no)	Age	49396.41333	2	24698.21	590.33**
	Error	6150.18	147	41.83796	
	Total	903953	150		
Female wing length (in cm)	Age	0.005889333	2	0.002945	2.57[NS]
	Error	0.168018	147	0.001143	
	Total	903953	150		

**P < 0.05 level; NS- Non significant.

Results

Males of *P. straiata* generally chose to mate with middle aged females more frequently than younger or older females (Table 1). In crosses involving young and middle aged females, in 32 of 50 trials, males mated with middle aged females, whereas in crosses involving older and middle aged females also in 34 of 50 trials males preferred to mate with middle aged females. However, younger female success occurred in 30 of 50 trials in crosses involving young and old females (Table.1).

The mean fecundity of young, middle aged, and old females of *P. straita* is given in Figure 1. It was noted that middle-aged females had greater mean fecundity than young or old females. One-way ANOVA followed by Tukey's honest *post hoc* test showed significant differences in mean fecundity among females of different age classes (Table 2). Middle aged females had significantly greater fecundity compared to younger and older females by Tukey's *post hoc* test .
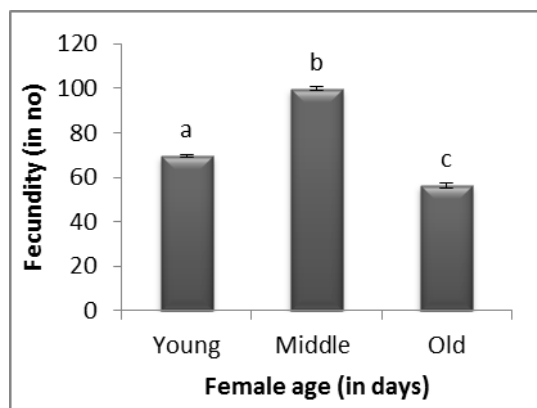

Figure 1. Age effect of female on fecundity in *P. straita*. (Different letters on the bar graph indicates significance at 0.05 level by Tukey's *post hoc* test).
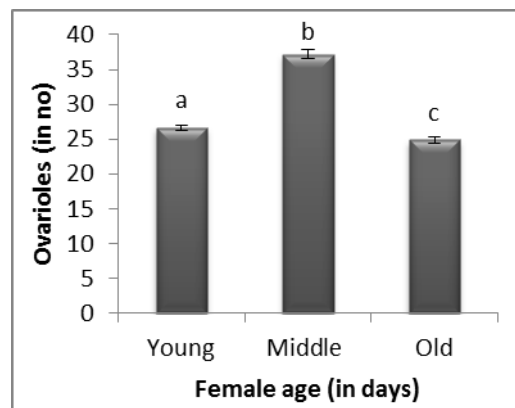

Figure 2. Age effect of female on ovariole number in *P. straita*. (Different letters on the bar graph indicates significance at 0.05 level by Tukey's *post hoc* test).
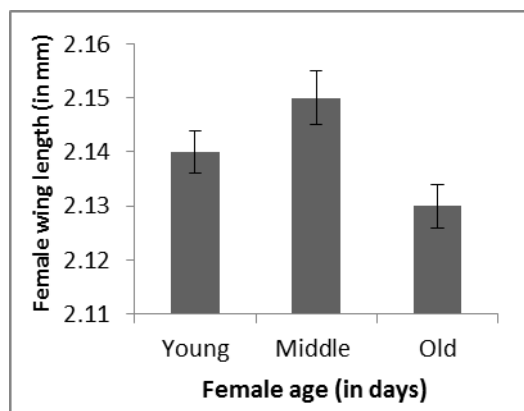

Figure 3. Age effect of female on wing length in *P. straita*. (Different letters on the bar graph indicates significance at 0.05 level by Tukey's *post hoc* test).

Figure 2 shows the mean number of ovarioles of young, middle-aged, and older females of *P. straita*. It was found that highest mean number of ovarioles was found in middle-aged females and was greater than young or old females. The mean number of ovarioles data were subjected to one-way ANOVA followed by Tukey's *post-hoc* test and showed significant variations in the mean numbers of ovarioles among females of different age classes (Table 2). Middle-aged females had a significantly greater mean number of ovarioles compared to young and old females by Tukey's *post hoc* test.

The mean female wing lengths of young, middle-aged, and old of *P. straita* are given in Figure 3. It was noted that differences in mean female wing lengths of different age classes were insignificant (Table 2).

Discussion

Restrictions in mating opportunities in males of *P. straita* may arise for various reasons, because, like males of *Drosophila* species, males of *P. straita* do not show parental care, and males only contribute sperm and components of the ejaculate to the courted female (Sharath Chandra and Hegde, 2003). In *Phorticella straita*, the high cost of reproduction is due to costs arising from such factors as energetically expensive courtship displays, the production of ejaculates, and time lost during different courtship display. It is assumed that in *P. straita* males exercise mate choice because 1) reproductive success of males of *P. straita* is limited by more factors than simply the number of females mates, and 2) females also differ in quality, *i.e.*, fecundity. These two criteria need to be in place for male *P. straita* mate selectivity to be evolutionarily advantageous. Table 1 reveals that males of *P. straita* prefer to mate with middle-aged females more frequently than young and old females, suggesting that males of *P. straita* exercise mate choice on the basis of female age. Thus, it appears that female age is an important determinant of male mate choice in *P. straita*. In other words, males of *P. straita* do not show the same levels of interest in females of different age classes they encounter. This confirms earlier studies of the existence of male mate choice for female age in other insects. Our results in *P. straita* also confirms work of Somashekar and Krishna (2011) and Prathibha and Krishna (2010), who, while working in *D. bipectinata* and *D. ananassae*, have also found that females of these species prefer to mate with middle aged females more frequently over young or old females. Gowaty *et al*. (2003), who, while working on *D. melanogaster*, also pointed out that males do not show the same level of interest in all females they encounter and provided evidence that males have also evolved to selectively mate. Therefore, these studies in species of *Drosophila* suggest that male preference for female traits may be an indirect way of assessing female fitness.

Like female mate preference even with male mate choice, it is difficult to separate among male choice, female-female competition, and differences in female motivations to mate (Byrne and Rice, 2006). Therefore, it is hypothesized that in *P. straita*, middle-aged females are more eager to mate than young or old females.

One theory suggests that when males seek direct fecundity benefits, they should discriminate among potential mating partners on the basis of traits which are reliable indicators of fecundity (Fitzpatrick *et al.*, 1995; Servedio and Lande, 2006). On the other hand, models of the evolution of male choice suggest that male choice tends to break down when male target arbitrary female traits rather than those that reliably signal fecundity (Kokko and Johnstone, 2002; Chenoweth *et al.*, 2006). Figure 1 and Table 2 show that in *P. straita* middle-aged females had significantly greater fecundity compared to younger and older females, suggesting that females of the same species show variations in reproductive potential across age. This confirms earlier work suggesting that female age is also one of the traits known to influence female fecundity in species of *Drosophila* (Prathibha and Krishna, 2010; Somashekar and Krishna, 2011).

Since the number of ovarioles is positively correlated with fecundity, we sacrificed females of different age classes of *P. straita* to study variations in the number of ovarioles if any at different female ages. Figure 2 and Table2 show that in *P. straita* middle-aged females had a significantly greater number of ovarioles than young and old females, and this follows a pattern of fecundity across female age. This again confirms earlier studies of the influence of female age on reproductive success in other insects (Bonduriansky, 2001). From the results, it was also noted that in *P. straita*, middle-aged females had a significantly greater number of ovarioles and higher fecundity compared to young and old females. Therefore, it is advantageous for males of *P. straita* to mate with middle-aged females compared to young and old females. However, it is not known why older females of this species had fewer ovarioles than young and middle-aged females. We do not know whether they reabsorb them as the females age or not.

Studies in insects have also found a positive correlation between female size and the number of ovarioles (Branquart and Hemptinne, 2000). Even in *Drosophila*, studies of Robertson (1957) found a positive correlation between female size and the number of ovarioles. Therefore, in the present study in *P. straita* female flies which were sacrificed to study the ovariole number were also used to measure wing length to understand relationships among female age, wing length, and ovariole number. It was found that there was an insignificant difference in the mean female wing length among females of different age classes (Figure 3 and Table 2). This suggests that in *P. straita* female age has no influence on female body size, but has a significant influence on ovariole number. These studies suggest that females of *P. straita* differ in reproductive potential,

i.e., fecundity and ovariole number across different female age classes, but they did not significantly differ in body size across the different female age classes. Therefore, one would expect that males of *P. straita* might exercise some degree of mate choice because of more factors that put an upper limit on male reproductive success. For this behavior to be adaptive, these individuals are expected to benefit by enhanced survival or fecundity. Therefore, it is important that males carefully select females to increase their fitness. The benefits may come in the form of direct enhancement of survival and fecundity (Trivers, 1972). Thus, these studies suggest males of *P. straita* discriminate their mate on the basis of age to obtain direct benefits.

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Biodiversity of Drosophilidae in Biligiriranga Hills wildlife sanctuary.

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Species of the genus *Drosophila* feed and breed on rotten fruits. They have been used as a model organism for over 100 years and thousands of scientists around the world work on it. This is because they are a highly tractable genetic model organism for understanding molecular mechanisms of human diseases. Many basic, biological, physiological, and neurological properties are conserved between mammals and *D. melanogaster*, and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly. However, taxonomic and population studies of these species have progressed little due to the lack of interest of people in this area. Therefore, very few studies have been undertaken to study *Drosophila* fauna from the field in India. Hence the present study has been undertaken in three places of Biligiriranga Hills wildlife sanctuary in Chamarajanagar District of south eastern Karnataka, India, to study *Drosophila* fauna.

In the present study the *Drosophila* fauna was collected from three different places in B,R Hills wildlife sanctuary, which is situated in Chamarajanagar District of south eastern Karnataka, South India, in November, 2014, using regular bottle trapping and banana bait methods. The study revealed a total of ten different species, namely *D. ananassae, D. bipectinata, D. kikkawai, D. malerkotliana, D. takahashii, D. neonasuta, D. varians, D. anomelani, D. sampangiensis,* and *D. nigra* being recorded. The frequency