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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 localities of Mysore, Karnataka, India and redistributed to twenty different culture bottles each with 40 flies (20 males and 20 females) containing wheat cream agar media. Here onwards this stock is designated as O population. These culture bottles were maintained at 21 ± 1°C at a relative humidity of 70% using 12:12 L:D cycle for three generations to allow them to acclimatize to the laboratory condition. These flies were used to collect eggs at the 4^{th} generation using Delcour's procedure (1969). Hundred eggs were seeded in a vial containing wheat cream agar medium. When adults emerged, virgin female and unmated males were isolated within three hrs of their eclosion and were aged individually in a vial containing wheat cream agar medium until they were used in the experiment. These flies were used to study female age influence on mating activities.

Selection of female age classes

The mating activities of females of different age classes were studied and found that female above 35 days will not show any courtship activities and she did not mate within 1 hr of observation. Therefore, in our study we assigned days for young, middle, and old aged female as follows [young age female (2-3 days), middle age female (17-18 days), and old age female (32-33days)].

Influence of female age on mating activities

A male with a female (younger, middle aged, or older) were aspirated into an Elens- Wattiaux mating chamber (1964), and observed for 1 h. We studied mating latency, courtship acts such as tapping, scissoring, vibrating, licking, circling, ignoring, extruding, and decamping, and copulation duration following the procedures of Hegde and Krishna (1997). One-way analysis of variance (ANOVA) followed by Tukey's honest post-hoc test was carried out on courtship activity data using SPSS vers 10.1 software (SPSS, Chicago, IL, USA).

Results and Discussion

Earlier studies in mating behavior of *Drosophila* have suggested that male activity and female receptivity are important for successful mating in *Drosophila* (Manning, 1961; Spieth, 1968). In *Drosophila* mating latency or courtship to copulation is a good estimate of sexual receptivity of females and sexual activity of males (Spieth and Ringo, 1983). During this period the male fly performs various courtship acts such as tapping, scissoring, vibration, circling, and licking to increase the receptivity of females (Spieth, 1968; Hegde and Krishna, 1997). Table 1 revealed that middle-aged females had taken shorter time for initiation of copulation and copulated longer when compared to either young or old aged females suggesting influence of female age on mating latency and copulation duration. As the speed is reverse of the time, flies which take more time were slow maters while those which take less time were fast maters. This suggests that in *D. ananassae* middle-aged females were fast maters while old age females were slow maters. This supports age influence mating activities in *D. pseudoobscura* (Noor, 1997). Since female receptivity is also related to time of initiation of copulation, therefore middle-aged females were more receptive than young or old aged females in *D. ananassae*.

We also quantified the male and female courtship activities such as tapping, scissoring, vibration, circling, licking, ignoring, extruding, and decamping (Table-1). It was noticed that males of *D. ananassae* showed greater courtship activities to middle-aged females compared to young or old-aged females' in turn middle-aged females showed less rejection activities to suggesting influence of female age on mating success in *D. ananassae.* This supports the earlier studies of age and other environmental factors influence on courtship activities in different species of *Drosophila*, too (Speith, 1952, 1968; Hegde and Krishna, 1997; Noor, 1997). Through these courtship activities, males of *Drosophila* convey chemical, auditory, and visual signals to middle-aged females better and try to convince the middle-aged female faster for mating than young or old-aged females. This agrees with earlier studies of *Drosophila* that males which perform greater courtship activities are better mates and obtained greater mating success than those males which do not show high level of courtship activities (Hegde and Krishna, 1997).

Table 1. Female age influence on mating activities in outbred population of *D. ananassae* (Values are Mean ± SE).

Parameters	Young (2-3days)	Middle (17-18 days)	Old (32-33 days)	F-Value
Mating latency (in minutes)	13.52 ± 0.62[b]	9.94 ± 0.38[a]	25.56 ± 0.1.89[c]	48.53**
Tapping	10.96 ± 0.35[b]	12.78 ± 0.33[c]	8.08 ± 0.30[a]	50.82**
Scissoring	10.82 ± 0.36[b]	11.82 ± 0.55[b]	8.36 ± 0.36[a]	16.57**
Vibration	10.12 ± 0.41[b]	11.32 ± 0.53[c]	8.24 ± 0.27[a]	13.63**
Circling	4.12 ± 0.20[b]	6.40 ± 0.28[c]	2.88 ± 0.18[a]	61.57**
Licking	3.58 ± 0.18[b]	5.32 ± 0.20[c]	2.54 ± 0.19[a]	52.26**
Ignoring	4.88 ± 0.15[b]	3.26 ± 0.19[a]	7.42 ± 0.25[c]	100.65**
Extruding	4.26 ± 0.22[b]	2.76 ± 0.14[a]	5.72 ± 0.31[c]	38.81**
Decamping	2.76 ± 0.13[a]	2.12 ± 0.14[a]	4.40 ± 0.29[b]	32.96**
Copulation duration (in minutes)	4.12 ± 0.04[b]	4.56 ± 0.06[c]	3.17 ± 0.04[a]	67.11**

**P< 0.001; Note: 1) Different letter in superscript in each row indicates significant by Tukey's test

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