

Age effect of male on male mating ability and progeny production in *D. melanogaster*

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Abstract: Age effect of male on male mating ability and progeny number has been studied in *D. melanogaster*. It was noticed that male at young age had inseminated more females and had produced greater number of progeny than middle aged and old males. Thus these studies suggest that in *D. melanogaster* remating of male increases male fitness. Further male at young age has greater fitness than middle and old aged males.

[Abolhasan Rezaei, M. S. Krishna. **Age effect of male on male mating ability and progeny production in *D. melanogaster***. *N Y Sci J* 2014;7(1):33-38]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 4

Keywords: male mating ability, *D. melanogaster*, male age, progeny number.

1. Introduction

All biological processes directly related to reproduction plays an important role in determining fitness. The fitness consists many components such as mating latency, mating time, duration between mating, fertility, fecundity, productivity, viability and longevity, etc. collectively, these processes determined the fertility component of fitness, where fertility is defined in its broadest sense (Turner and Andersson, 1983). The sexual behaviors of various species of *Drosophila*, with particular reference to their basic courtship patterns, genetic control, role of stimuli, and contributions of the 2 sexes to variations in mating activity and repeated mating, have been extensively studied (Parsons, 1973, Banerjee and Singh, 1977; Gromko and Pyle, 1978; Casares et al., 1998). The role of fertility differences to selection has been demonstrated repeatedly in different species of *Drosophila* (Turner and Andersson, 1983). One aspect of fertility that has only recently received attention only recently is multiple mating (review of *Drosophila* research in Gromko et al., in press). Once thought to be mostly a laboratory phenomenon in *Drosophila*, multiple mating has been reported in natural populations of several species (reviewed in Gromko et al., in press; Loukas et al., 1981), and at very high rates (e.g., Levine et al., 1980). Rates of multiple insemination sometimes differ among populations of a species, as for example they do among the three populations of *D. pseudoobscura* studied (Anderson, 1974; Cobbs, 1977; Levine et al., 1980).

Remating is common in many species of *Drosophila* under both natural and laboratory conditions. Multiple mating in *Drosophila* bears a direct relation to fitness (Koref- Santibanez, 2001). Remating time of male and female in different species of *Drosophila* has been studied. (Singh and Singh, 2002; Karif-Santibanez, 2001). Remating and sperm storage are specific features that can play important

roles in determining female fecundity, male mating success. Remating reduces the maternal survival (Levine et al., 1980; Partridge and Farquhar, 1981; Nicholas et al., 2008). Markow et al., (1978) have found that females are able to distinguish between mated and unmated males and those that had a previous mating experience, showing preference for virgin males. These results are not surprising, since multiple insemination may be sensitive to both population densities and environmental conditions.

It has been widely demonstrated that males of different species of *Drosophila* can inseminate more than 1 female, and the mating ability of males is influenced by genetic and other factors (Prakash, 1967; Singh and Chatterjee, 1987; Sanchez Prado and Blanco Lizana, 1989; Singh and Singh, 1999). According to Gromko, (1992), multiple mating is widely believed to be advantageous to males, and selection by males can produce a correlated response in females. In *Drosophila* and other organisms, the importance of male size in courtship and mating has also been studied (Hegde and Krishna, 1997).

Age in male insects may effect reproductive activities including success in intersexual encounters, sperm procedure and female fecundity. However, the importance of male age on sexual behavior is not straight forward, and in most cases, only 1 or a few aspects of male fitness like success in intermale competition, fecundity, fertility, and longevity have been studied (Somashekar and Krishna, 2011). In fact, male remating is also a strategy for improving the fitness of individuals of polygamous populations. This is because in species of *Drosophila* in each mating, male transfers ejaculatory substances which includes accessory gland proteins and sperms to the mated female. Acps in mated female includes egg laying or oviposition and also facilitates fertilization of sperm into the eggs. Therefore males inseminating more females contribute more progenies to its next

generation compared to the male which inseminate less females. In *Drosophila*, the influence of female remating on fitness has been studied (Sisodia and Singh, 2004). The further influence of male size on male remating components has been largely ignored. Therefore the present investigation has been undertaken in *D. melanogaster* to study age effect of male on mating ability and progeny production.

2. Materials and Methods:

Progenies of 50 isofemale lines of *D. melanogaster* collected at Mysore, India were mixed together to establish outbred population. Twenty males and twenty females per bottle were cultured at $22^{\circ}\text{C}\pm 1^{\circ}\text{C}$ with a relative humidity of 70% using wheat cream agar medium in 12L:12D cycle for 3 generations to the acclimatize to laboratory condition. At 4th generation, eggs were collected from this stock using Delcour's procedure (Delcour, 1969). Eggs (100) were transferred separately into a vial containing wheat cream agar medium. When adults start emerging from these vials, virgin females and unmated males were isolated within 3 hours of their eclosion and were aged as required for the same laboratory condition.

Longitudinal study includes the analysis of same individual male at young, middle and old age. To study this 2-3 days old male along with virgin of 5-6 days old female were introduced into a mating chamber and observed for an hour. If the pair did not mate within an hour, it was discarded, if mating occurred mate female was individually aspirated into a new vial containing wheat cream agar medium for every 24hrs. This was continued until the death of the female. The progeny emerged from the eggs of mated female was recorded. The mated male was allowed to mate with a 2nd virgin female (5-6d old). If mating occurred with the 2nd female, they were allowed to complete copulation and mated female was transferred to new vial to record progeny number as described above. This process was repeated, and number of females inseminated by each male in 1hr and progeny produced from them was recorded as the male mating ability.

Soon after recording male mating ability for 2-3 days the mated male was individually aspirated back to the new culture and allowed for ageing (27-28 days). When this male attained 27-28 days, they were individually used to record male mating ability and progeny number as described above. After recording this, once again mated male was aspirated again into a new culture vial containing wheat cream agar medium and allowed them to age (52-53d old). When they attained the age of 52-53 days, these mated males were individually subjected for analysis of male mating ability and progeny number as described above. Fifty trials were continued separately for both outbred and Conton-S populations. One way ANOVA followed by Tukey's post hoc test was carried out on male mating ability and progeny number. General Linear Model (repeated measures) has also been applied on male mating ability and progeny number.

3. Results

The mean male mating abilities at young, middle and old age of outbred and Conton-S experimental stocks of *D. melanogaster* are given in figure 1. The outbred strain inseminated more females compared to that of Conton-S stocks. In both the stocks young males at age had inseminated more females compared to males at middle and old age. The mean male mating ability data subjected to one-way ANOVA followed by Tukey's post hoc test showed significant differences in the mean male mating ability among the 2 strains, among males of the 3 age classes, and also interactions stocks and male age (Table 1). Tukey's post hoc test showed that males of the Conton-S stocks had inseminated significantly fewer females compared to males of the outbred stocks.

The results of the GLMs (repeated measures) age and male mating ability with the 1st and 2nd females are provided in Table 2 and figure 1. A negative relation between the male age and male mating ability and also between male age and progeny number suggests that males at young age inseminated more females in a given unit a time and produced greater number a progeny than those of males at middle aged and old age (Figure 2, Tables 3 and 4). The result was found to be similar in both the experimental stocks.

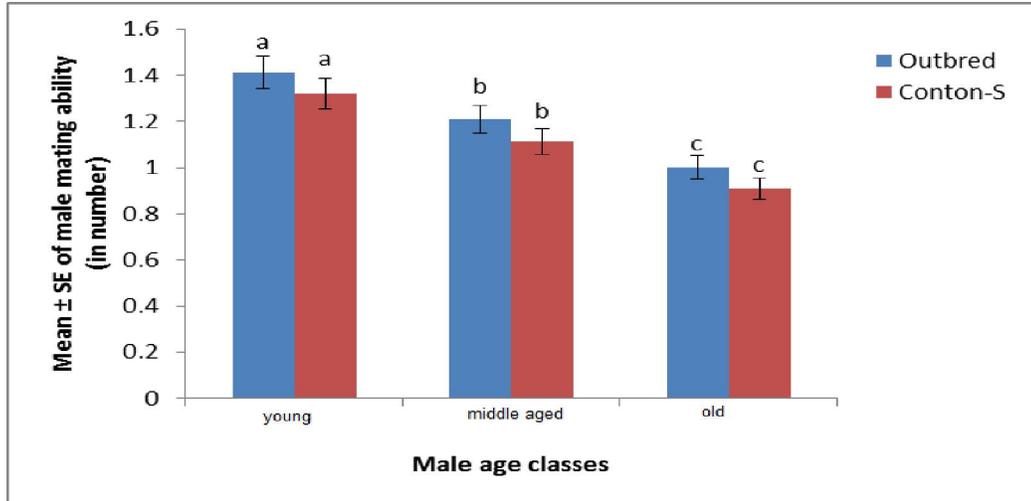


Figure 1: Age effect of male on male mating ability in *D. melanogaster*.

Table 1. One way ANOVA of male mating ability in *Drosophila melanogaster*

Experimental stocks	Parameter	Source	Type III Sum of Squares	df	Mean square	Sig.
Outbred	Male mating ability	Age	6.253	2	0.161	19.459**
		Error	23.620	147		
		Total	29873.12	150		
Conton-S	Male mating ability	Age	6.212	2	0.154	18.459**
		Error	23.611	147		
		Total	29873.00	150		

** Significant at $p < 0.001$.

Table 2. General linear model (repeated measures) between male age, male mating ability of *D. melanogaster*.

Experimental Stocks	Parameter	Type III Sum of Squares	df	Mean square	F
Outbred	Source male mating ability	6.250	1	6.250	42.241**
	Male mating ability × male age linear	0.003	1	0.003	0.017**
	Error (male mating ability) linear	7.250	49	0.250	
Conton-S	Source male mating ability	5.251	1	4.213	41.040**
	Male mating ability × male age linear	0.001	1	0.001	0.011**
	Error(male mating ability) linear	6.250	49	0.212	

** Significant at $p < 0.001$.

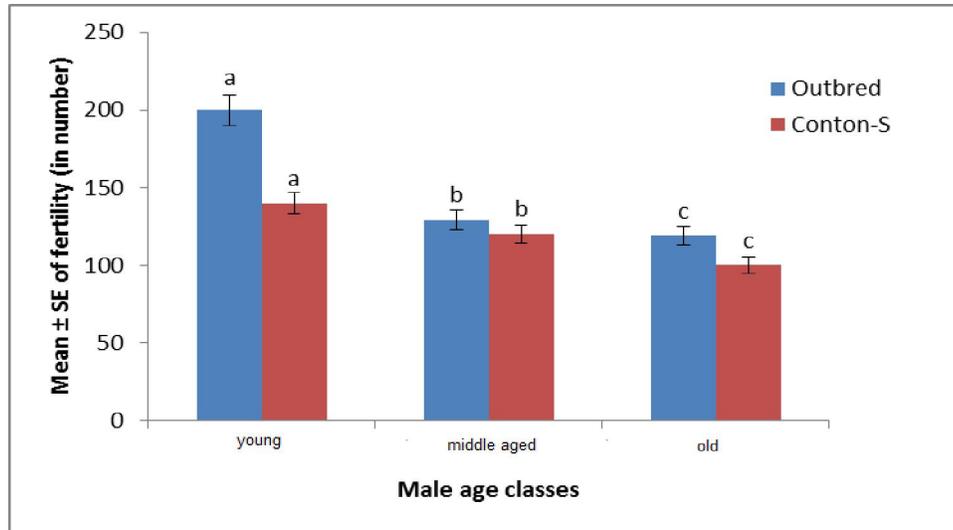


Figure 2: Progeny production of female mated with male at young, middle and old age of *D. melanogaster*.

Table 3. One way ANOVA of fertility in *Drosophila melanogaster*

Experimental stocks	Parameter	Source	Type III Sum of Squares	df	Mean square	F-Value
Outbred	Fertility	Age	326727.213	2	163363.607	42.825**
		Error	560751.560	147	3814.636	
		Total	46390343	150		
Conton-S	Fertility	Age	312727.210	2	163323.124	42.235**
		Error	560231.521	147	3802.213	
		Total	46391221	150		

** Significant at $p < 0.001$

Table 4. General linear model (repeated measures) between male ages and fertility of *D. melanogaster*.

Experimental Stocks	Parameter	Type III Sum of Squares	df	Mean square	F
Outbred	Source fertility	265946.490	1	265946.490	65.477**
	Fertility × male age linear	57713.070	1	57713.070	17.868**
	Error (fertility) linear	199021.010	49	4061.653	
Conton-S	Source fertility	265923.125	1	265923.412	65.412**
	Fertility × male age linear	547112.071	1	54711.071	20.345**
	Error (fertility) linear	199121.12	49	4012.321	

** Significant at $p < 0.001$

4. Discussion

Reproductive capacity is particularly a good index of fitness in organisms that go through repeated cycles of rapid population growth and it has evolved as a way for species to maximize their potential for survival. Reproductive success of females depend on number of eggs laid and male reproductive success depends of the number of females he could inseminate. Fecundity is the most obvious trait that influences the reproductive ability of female and is usually considered as female fitness component, and it has been analyzed in different species of *Drosophila*. It shows that fecundity is influenced by mating flies, age, body size, environmental factors, genotype and also fecundity

enhancing substances (FES). The fecundity of virgin females is more when compared to mated female (Partridge et al., 1986; Gillot, 2003; Roopashree et al., 2001).

The primary function of mating is the transfer of sperm to females, since each mating offers an opportunity to father offspring. Males can generally increase their fitness by mating with many mates, and high mating rates are thus typically associated with high male reproductive success. Males of *D. melanogaster*, like most male insects, provides no parental care to offspring following female insemination (Thornhill and Alcock, 1983). Male fitness is therefore related to the number of females

inseminated and prevention of females from re-mating (Parker, 1970; Jones and Elgar, 2004; Jones et al., 2007). In the present study, mating ability of male at young, middle aged, old age was studied in two experimental stocks of *D. melanogaster* (Fig. 2, Table 3 and 4). It was noted that the outbred stock had inseminated more number of females in a given unit of time than Conton-S stock. This shows the existence of interstocks variation in male mating ability. This confirms findings of an earlier study on interpopulational variations in male mating ability in *D. bipectinata* (Guru Prasad et al., 2008). Since the male's mating ability is a fitness character, the observed variations in male mating ability among the different geographic populations experimental stock suggest interactions of these stocks with heterogeneous environments. Among the two experimental stocks studied, the outbred stock had produced greater progeny number than the Conton-S. It was also noticed females that mated with males at young age had produced greater number of progeny than females that mated with either middle aged or old males. Our study supports widely accepted view that males which inseminate more females produces more progeny than those that of males that inseminate less number of females (Gromko, 1992). Our study also confirms the earlier works that male remating increases male fitness in different species of *Drosophila* (Singh and Singh, 2000; Krishna and Hegde, 1997; Curu Prasad et al., 2008). Thus these studies in *D. melanogaster* male at young age had inseminated more females and had produced greater number of progeny than male at middle and old age.

Acknowledgement:

The authors are grateful to the Chairman, Department of Studies in Zoology, University of Mysore, and *Drosophila* stock center, University of Mysore for providing facilities to carry out this work.

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References:

1. Anderson WW. Frequent multiple in-semination in a natural population of *Drosophila pseudoobscura*. *Amer Natur* 1974;108, 709-711.
2. Banerjee R, Singh BN. Population and behaviour genetics of *Drosophila bipectinata*. *Proc. Indian Nat Sci Acad.B* 1997; 63: 399-410.
3. Bonduriansky R. The evolution of male mate choice in insect: a synthesis of ideas and evidence. *Biological reviews of the Cambridge philosophical society* 2001; 76: 305-339.
4. Casares P, MC Carracedo, B Del Rio, R Pinerio, L Garcia-Florez, Barros AR. Disentangling the effects of mating propensity and mating choice in *Drosophila*. *Evol* 1998; 52: 126-133.
5. Cobbs G. Multiple inseminations and male sexual selection in natural populations of *Drosophila pseudoobscura*. *Amer Natur* 1977; 111: 641- 656.
6. Delcour J. A rapid and efficient method of egg collecting. *Dros Inf Ser* 1969; 44: 133-134.
7. Jones TM, Elgar MA. The role of male age, sperm age and mating history on fecundity and fertilization success in the hide beetle. *Proc Roy Soc Lond B Biol* 2004; 271: 1311-8.
8. Jones TM, Featherston R, Paris D, Elgar MA. Age-related sperm transfer and sperm competitive ability in the male hide beetle. *Behav Ecol* 2007; 18: 251-8.
9. Hegde SN, Krishna MS. Size assortative mating in *Drosophila malerkotliana*. *Anim Beh* 1997; 54: 419-426.
10. Gillott C. Male accessory gland secretions: Modulators of Female Reproductive Physiology and Behavior. *Annu. Rev. Entomol* 2003; 48: 163-184.
11. Gromko MH. Genetic correlation of male and female mating frequency: evidence from *Drosophila melanogaster*. *Anim. Behav* 1992; 43: 176-177.
12. Gromko MH, DW Pyle. Sperm competition, male fitness and repeated mating by female *Drosophila melanogaster*. *Evol* 1978; 32: 588-593.
13. Gromko MH, D G Gilbert and Richmond R C. Sperm transfer and use in the repeat mating system of *Drosophila*. In R. L. Smith (ed.), *Sperm Competition and the Evolution of Animal Mating Systems*. Academic Press, N.Y. In press.
14. Guru Prasad BR, SN Hegde, MS Krishna. Positive relation between male size and remating success in some population of *Drosophila bipectinata*. *Zool Stud* 2008; 47: 651-659.
15. Koref- Santibanez S. Effects of age and experience on mating activity in the sibling species *Drosophila pavani* and *Drosophila gaucha*. *Behavior Gene* 2001; 31: 287-297.
16. Levine L, M Asmussen, O Olvera, JR Powell ME Delarosa, VM Salceda, MI Gaso, J Guzman, and Anderson WWW. Population genetics of Mexican *Drosophila*. V.A. high rate of multiple inseminations in a natural population of *Drosophila pseudoobscura*. *Am Nat* 1980; 116: 493-503.
17. Levine L, M Asmussen, O Olvera, JR Pow- Ell, ME, Delasora V M, Salceda M I, Gaso J, Guzman, and Anderson WW. Population genetics

- of *Mexican Drosophila*. V. An extremely high rate of multiple insemination in a natural population of *Drosophila pseudoobscura*. *Amer Natur* 1980; 116: 493-503.
18. Markow TA, M Quaid, Kerr S. Male mating experience and competitive courtship success in *D. melanogaster*. *Nat* 1978; 276: 821-822.
 19. Nicholas KP, FG Laura and A Deborah, Roach. Mating frequency and inclusive fitness in *Drosophila melanogaster*. *The American naturalist* 2008; 171: 10-21.
 20. Parsons PA. Behavioral and ecological genetics: a study in *Drosophila*. Oxford, UK: Clarendon Press 1973.
 21. Parker GA. Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 1970; 45: 525-567.
 22. Prakash S. Association between mating speed and fertility in *Drosophila robusta*. *Gene* 1967; 57: 655-663.
 23. Partridge L, and Farquhar M. Sexual activity reduces lifespan of male fruit flies. *Nat* 1981; 294: 580-582.
 24. Partridge L, K Flower, S Trevitt and Sharp W. An examination of the effects of males on the survival and eggproduction rates of *Drosophila melanogaster*. *J Insect Physiol* 1986; 32: 925-929.
 25. Roopashree S, K Ravi Ram, and Ramesh SR. Genotype environment interaction and fecundity in *Drosophila*. *Dros inf serv* 2001; 84: 93-95.
 26. Somashekar K, Krishna MS. Evidence of female preference for older males in *Drosophila bipunctinata*. *Zool Stud* 2011; 50: 1-15.
 27. Singh BN, S Chatterjee. Variation in mating propensity and fertility in isofemale strains of *Drosophila ananassae*. *Gene* 1987; 73: 237.
 28. Singh BN, SR Singh. Mating success in *Drosophila ananassae*: evidence for greater variation in receptivity of females compared to male mating ability. *Curr Sci* 1999; 77: 1200-1203.
 29. Singh SR, Singh BN. Male remating in *Drosophila ananassae*: evidence for interstrain variation in remating time and shorter duration of copulation during second mating. *Zool Sci* 2001; 17: 389-393
 30. Sisodia S, Singh BN. Size dependent sexual selection in *Drosophila ananassae*. *Gene* 2004; 121: 207-217.
 31. Sanchez Prado JA, Blanco Lizana G. Mating patterns of different Adh genotypes of *Drosophila melanogaster*. I. differences in mating ability. *Gene* 1989; 78: 219-224.
 32. Singh SR, BN Singh and Hoenigsberg HF. Female remating, sperm competition and sexual selection in *Drosophila*?. *Gene Mol Res* 2002; 1(3): 178-215.
 33. Turner ME and Anderson WA. Multiple mating and female fitness in *Drosophila melanogaster*. *Evol* 1983; 37: 714-723.
 34. Thornhill R, Alcock J. The Evolution of Insect Mating Systems. *Nat* 2001; 564 pages.

12/29/2013