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The effect of aging on the female reproductive performance in *Drosophilamelanogaster*

Fatemeh Tavakkoli
1- Drosophila stock center, Department of Zoology-Mysore University, India
Email: fateme_tavakkoli84@yahoo.com.

ABSTRACT

Female age influence on male reproductive performance and also female fitness traits. The male recorded such as courtship activities, mating latency, copulation duration, and female fitness traits, such as, fecundity, has been used from isofemale lines of *Drosophila melanogaster*. The female age including, 2-3 days aged old (young aged), 17-18 days aged old (middle aged) and 31-32 days aged old (old aged) mated with virgin male (5-6 days aged old) that all of the flies were exposure of middle temperature (22°c). The results of SPSS software analyzed that in almost all of experimental young aged old had significantly level than middle and old age. Courtship activities elements, in young age female were greater than middle and old age, when mated with young age male. This variability suggests that parental age effected on the male reproductive performance and female fitness traits. Therefore, the female age specific possibility occurrence on reproductive access as follows by: young aged> middle aged>old aged.

Keywords: *Drosophila melanogaster*, female fitness traits, male age reproductive performance, longitudinal studies.

INTRODUCTION

In most species of insects aging is one of the extreme factors that influence mating behavior and reproductive success (Delisle 1995; Jones *et al*. 2000; Moore and Moore 2001). Aging is a principle process such longevity study have led to the male and female reproductive performance. (Luckinbill *et al*. 1984; Rose 1984; Partridge and Fowler 1992; Zwaan *et al*. 1995; Partridge and Barton, 1996). Furthermore, age effect is important on the male mating success and among of female fitness traits such as fecundity, fertility, courtship activities and longevity in *Drosophila melanogaster* (Anderson *et al*., 1973; Brittnacher, 1981).

Hansen and Price 1995; Brooks and Kemp 2001, proposed, that age are often regarded as an important factor for mate choice. The aging cause of decreasing or increasing viability and fertility might be contributed to a negative relationship between age and mate quality. Female fitness traits and male reproductive performance may also be significantly penetrated by age. (Moore and Moore 2001; Torres-Vila *et al*. 2002; Pervez *et al*. 2004; Kruger 2005; Omkar *et al*. 2006; Wenninger and Averill 2006; Maklakov *et al*. 2007). In *Drosophila melanogaster* the effect of male age on the female fitness traits also were studied by (Kokko and Lindström 1996; Kokko 1997; Brooks and Kemp 2001; Proulx *et al*. 2002; Rose and Charlesworth 1980; Rose 1984; Partridge and Fowler 1992; Zwaan *et al*. 1995; Promislow *et al*. 1996; Nuzhdin *et al*. 1997; Leips and Mackay 2000).

They were shown that female preferences with respect male age differs. In

Drosophila female preferences for older males, because the reliability of male signaling increases with age and they have proven survival ability (Avent *et al*. 2008). In opposite of that other studies showed middle and young male age were favored and highest breeding values for fitness, and thus are higher genetic quality than older males(Martin *et al*. 2003; Hansen and Price, 1995). These experimental results of age-based mate preference suggests that *Drosophila* species have developed different mate preference strategies to enhance their reproductive success. However, there are a few experiments that female age mate prefer on the male age. The same study had done by Jones *et al*., 2000, that females of *sandflies* prefer middle than male young and older age, accordance of these studies performed by Chapman *et al*., 1995 and Wolfner 1997, they suggested that parental age effects may influence quality directly, through factors in the sperm pronucleus, or indirectly, male influence of female in during of mating, may also be in male influenced on female physiological reproductive performance such as quantities and qualities of sperm (Trivers 1972).

Hence the female age is an important factor for determining among of reproductive performance and reproductive success. By concluding these studies, I want to consider age-based on the reproductive performance. Firstly, we assessed whether, when presented with a choice of young, middle-aged, and old age females, males of show different mating preference with respect to female age. Secondly, we performed a further experiment in which pairings were established using different aged males and females. Then we compared the courtship activities, female and male longevity of varying age paired to males of different age classes.

MATERIALS AND METHODS

a) Establishment of experimental stock:

The isofemale lines of *Drosophila melanogaster* collected from the national *Drosophila* stock center, Department of Zoology(UOM)- India. These flies were used to study the effects of female reproductive performance on the male fitness traits. These flies were cultured and using the wheat cream agar medium at 22℃±1℃ and 70% relative humidity. In each generation flies were mixed together and 40 flies (20 males and 20 females) , for three generations. At fourth generation virgin females and males were isolated and these were used in the present study.

b) Selection of female age-classes:

When adults emerged, virgin females and unmated males were isolated within 3 h of eclosion and were aspirated into a new vial containing wheat cream agar medium. These flies were aged as required for the experiment. In order to provide uniform environmental conditions for females of different age classes, female flies collected first were assigned to 31-32d (older); females fly collected next were assigned to 17-18 d (middle-aged). Following this, female flies collected were assigned to 2-3 d (younger).

D. The influence of female age on male mating activities:

Females belonging to a particular age-group young age (2-3 days), middle age (17-18 days) and old age (31-32) were allowed to mate with the male age (5-6 days) by aspirating one of each type into a glass vial using an aspirator. The observation was made for 1 hour. If mating does not occur within 1 hour it was replaced by new pair. A total 25 trials were made for each female age-class. The mating latency which refers to the time gap between the introduction of the flies till the initiation of mating

and copulation duration which refers to the time gap between the initiations of copulation to the termination of copulation, was noted down following the procedures of Hegde and Krishna (1997). The other male courtship activities such as tapping, scissoring, vibration, licking, circling, ignoring, extruding, and decamping, following the procedure of Hegde and Krishna (1997). These courtship acts are described as follows:

Tapping: The male initiates courtship with a foreleg motion partially extending and simultaneously elevating one or both forelegs, followed by a downward striking motion, thus bringing the ventral surface of the tarsus in contact with the partner.

Scissoring: The courting male opens and closes both wings with a scissor like movement during the interval between wing vibrations.

Vibration: The male expands one wing laterally from the resting position, and then moves one or both wings rapidly up and down.

Licking: The courting male positions himself closely behind the female, extends his proboscis, and licks her genitalia.

Circling: After posturing at the side or rear of a non-receptive female, the male faces the female as he moves about. Mean values were calculated and analyzed. The male may move to face her and then retraces his path to the rear, or may at other times move completely around her in a circle.

Ignoring: The non-receptive female simply continues with whatever activity in which she was previously engaged, apparently ignoring actions by the male.

Extruding: The non-receptive female presses the vaginal plates together, contracting certain abdominal muscles and apparently relaxing others.

Decamping: The non-receptive female attempts to escape by running, jumping, or flying away from the courting male. Two different observers recorded the behavior of the male and female simultaneously for one hour. The number of pairs mated was also recorded.

The statistical programs. One -way ANOVA and Tukey's honest post hoc tests were used on mean data of mating latency, courtship activities, copulation duration, and female longevity by using SPSS 10.1 software.(1999).

RESULTS

Male age influence on female courtship activities:

The male courtship activities such as, mating latency, courtship activities, tapping, scissoring, circling, vibrating, licking on the female age classes including, young (2-3 days aged female), middle (17-18 days aged female) and old (31-32 days aged female) analyzed by SPSS software. The mean value of the results is shown regarding mating latency and copulation duration, the length of the graph when male (5-6 days aged) mated with 2-3 days aged female longer than 17-18 days and 31-32days aged female. Figure 1 shows the mean value of male courtship activities contains, tapping, scissoring, circling, vibrating and licking in middle female age was more than young and old age female, The results as follows, middle age > young age > old age female (Figure 1).

Female courtship activities: The mean value of female courtship activities such as decamping, ignoring and extruding, were analysed and shows in figure 2. The female young age when mated with 5-6 days aged male , the length of graph longer than middle and female old age . The results as follows: young>middle >old female age classes.

Male mating latency and copulation duration: The male mating latency, from the time of initiation until copulation and copulation duration means, time of initiation copulation until end of copulation were recorded between three female age classes (young, middle and old). In figure 3, shows that the length of graph in young female longer than middle and old female.

Longevity experimenters: In the present study were used three female age classes, (young, middle and old) for longitudinal studies. In related to young female were mated with 5-6 days aged male, after finishing egg laid female were kept in the vial contain wheat cream medium agar until dead. The mean value of the result between females are shown, young female age more than middle and old age female, In figure 4 also are shown, longer of graph in old female longer than middle and young age female. The result of one way ANOVA also in table 1 showed, there was a significant variation between courtship activity parameters, except of tapping, including, scissoring, licking, vibrating, circling. Regarding female courtship activities, including, decamping, ignoring and extruding had a significant variation between elements. Mating latency and copulation duration also were compared to one way ANOVA, it was noticed that a significant variation in mating latency and copulation duration. The female longevity also were compared, it was noticed that there was a significant variation between three female age classes.(Table 1).

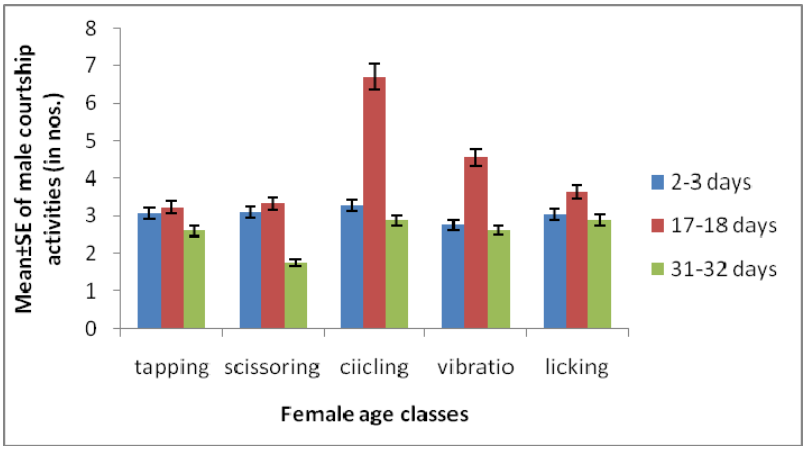

Fig.1: Male courtship activities are shown, there was a significant variation between three female age classes. (Young, middle and old).

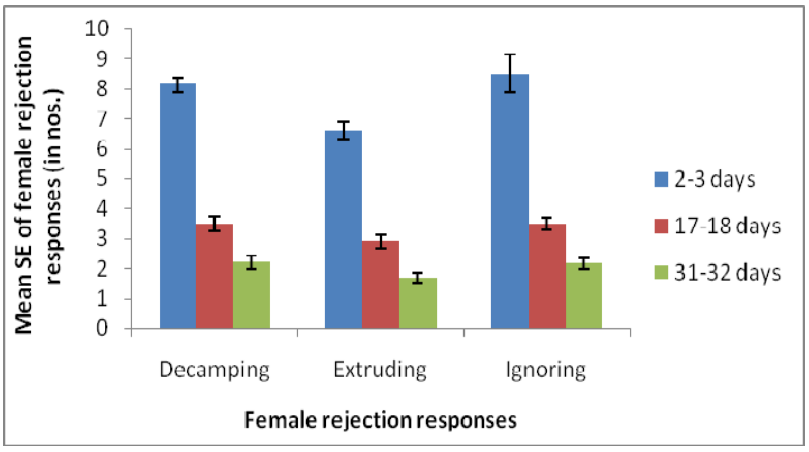

Fig. 2: The result of female courtship activities are shown, there were a significant variation between three female age classes. (Young, middle and old).

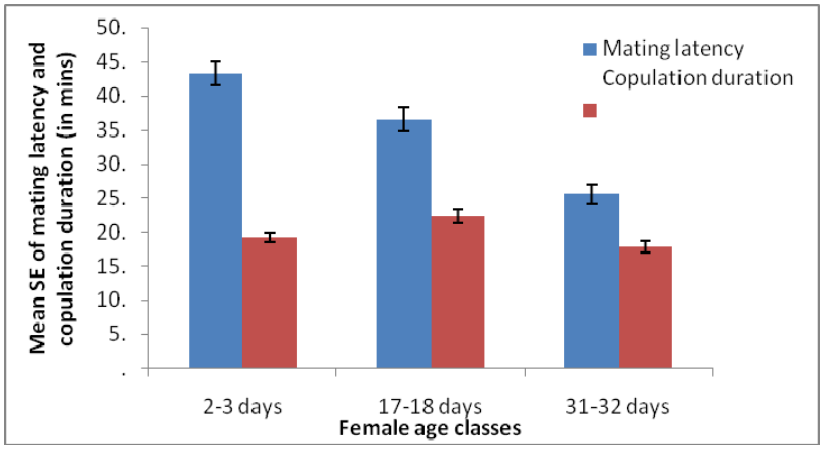

Fig. 3: The result of male courtship activities are shown, there were a significant variation between three female age classes.(Young, middle and old).

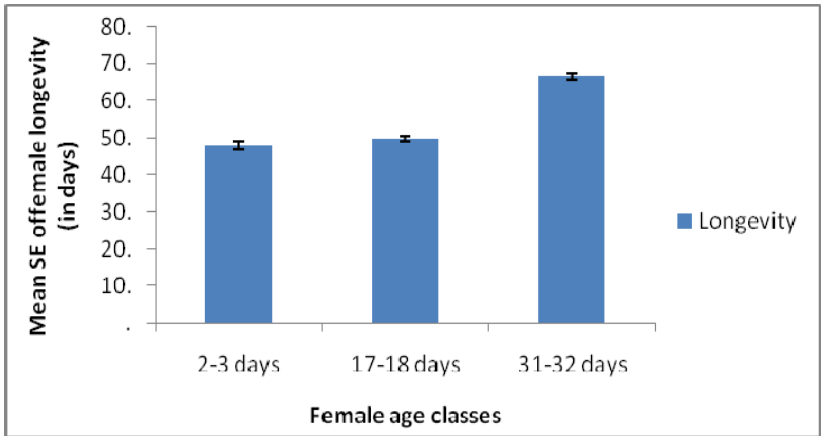

Fig. 4: The result of female longevity studies. Results are shown, there were a significant variation between three female age classes.

Table 1: The result of mean value courtship activities parameters and longevity, mating latency and copulation duration are shown, regarding all of results young female age were more than middle and old age.

Source (age)	Dependent variable	Type of III sum of squares	df.	Mean square	F value	Significant
Between groups	Mating latency	15534.17	2	7767.090	30.069	0.001*
Between groups	Copulation duration	1119.847	2	559.923	7.456	0.001*
Between groups	Longevity	20899.67	2	10449.836	145.271	0.001*
Between groups	Tapping	19.787	2	9.893	2.603	0.076^{ns}
Between groups	Licking	35.043	2	17.521	2.086	0.0126*
Between groups	Circling	890.981	2	445.490	30.167	0.001*
Between groups	Vibrating	220.527	2	110.224	10.370	0.001*
Between groups	Scissoring	150.444	2	75.222	21.020	0.001*
Between groups	Ignoring	2379.969	2	1189.985	74.503	0.001*
Between groups	Decamping	1879.782	2	939.891	152.563	0.001*
Between groups	Extruding	1278.976	2	639.488	117.861	0.001*

*P value <0.05 and NS,means non significant.

DISCUSSION

Benefits of based aged on the courtship activity and reproductive performance:

We explore that the beneficial trait will be transferred to male middle and young age. So, we descriptive why exactly females paired to male young and middle age,

and produce eggs with higher hatching success. Results have shown a positive correlation response to selection for age at reproduction, so, were observed a difference in the proportion of eggs hatching between the experiments, the results of the ANOVA are shown that among of fecundity and fertility in male young age more than middle and old age. Also observed that both maternal traits and paternal traits can influence on the fecundity parameters. Therefore there were increased among of fecundity between female and male mated young age, however the results of the ANOVA are shown there are significant variances between young, middle and old age related to fecundity elements, but also the results of graphs were observed that the length of graph between male young age and old is very high and between young and middle was medium, also between middle and old was medium.

These experiments denotes there were positive correlations between young age and among egg laid, but there are negative correlation between old age, among of egg laid. These results suggest that, difference in reproductive rate are an explanation of the effect of age on the reproductive performance had a remarkably different effect on offspring viability between mated males, here we showed the age effected on the reproductive performance, however in a study by Jones *et al*., (2000) are shown that females of *sandflies* prefer middle aged than young and old aged but the males had age from zero to two, five to six and eight to ten days aged while we done on male of 5-6 days aged, also Hansen and Price predicted that older males should be less attractive. Hanson and Price also argument the lower hatching success of females mating with old males is hard to explain with viability indicator models. But suggested that older females are less well adapted have accumulated germ-line mutations, or have trade - offs late reproduction against early survival (Hansen and price, 1995).

Male age based on the courtship activity:

The parameters of courtship activity, including, tapping, wing vibration, licking, decamping, scissoring, extruding , ignoring, and male mating ability was discussed for three female age classes (2-3, 17-18 and 31-32 aged). We studied courtship activity parameters with ANOVA analysis, so, post hoc analysis indicated young, middle and old aged significantly variation in copulation duration and mating latency, hence, the lack of age effect on the offspring / female fitness traits are important. In addition, there some evidence that male age influence on the copulation time. For example, female red-winged *blackbirds* typically select older males for extra pair copulations (Weatherhead and Boag, 1995; see Grant and Grant, 1987; Dickinson, 2001), also in a *sandfly*, females favor middle-aged males (Jones *et al*., 2000).

Results of fecundity were shown, in female young age among of fecund more than middle and old age, perhaps the time of copulation is effective on the rate of egg laid, and causing increased damage to females, moreover (Blanckenhorn *et al*., 2002) has shown that increase of copulation duration cause of decrease female's longevity, so it is possible that longer copulations damage female's ability (Blanckenhorn *et al*., 2002). Similarity age influencing on mating ability are reported in *D. pseudobscura*. In addition other parameters of courtship activity were examined. The courtship activity for successfully mating, male activity and female receptivity are important (Manning 1961).

Moreover the courtship activity and mating behavior, in flies consist of a repertoire of specific signals delivered by the male and the female only during the mating event. That these signals may play in the recognition and sexual selection within the species (Spieth and Ringo 1983, Spiess 1987). In *Drosophila* species, signals delivered during the mating behavior can be vibration, scissoring, decamping,

licking, extruding, ignoring the parameters (Spieth 1968; Hegde and Krishna 1997). Our experiments about mating behavior such as, circling, tapping, licking, vibration, scissoring, decamping, analyzed by one way-ANOVA analysis in table 1, results revealed that young, middle and old age female, were significant. These results suggested that age influencing mating activities, had positive and negative variation between age classes and mating behavior. Similarity in figure 1, were observed, females of young age more mating activity than middle and old age, so, the young age are better mates and obtain good genes than those males which was not observed high levels of courtship activity.

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REFERENCES

Anderson, W.W. (1973). Genetic divergence in body size among experimental populations of *Drosophila pseudoobscura* kept at different temperatures. Evolution, 27: 278–284.

Avent T.D, Price Tar, Wedell N. (2008). Age based female preference in the fruit Fly *D. pseudoobscura*. Animal behavior. 75 (4): 1413-1421.

Brittnacher, J. G. (1981). Genetic variation and genetic load due to the male reproductive component of fitness in *Drosophila*.Genetic.97:719-730.

Blanckenhorn, W. U., Hosken, D. J., Martin, O. Y., Reim, C., Teuschl, Y. and Ward, P. I. (2002). The costs of copulating in the dung fly *Sepsis cynipsea*. Behav. Ecol., 13:353–358.

Brooks, R. and Kemp, D. J. (2001). Can older males deliver the good genes?.Trends in Ecology and Evolution.16: 308-313.

Chapman, T., L.F. Liddle., J.M. Kalb., M.F. Wolfner., Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature. 373: 241–244.

Delisle J. (1995). Effect of male and female age on the mating success of the obliquebandedleafroller *Choristoneurarosaceana* (Lepidoptera: Tortricidae) under different ecological conditions. J Insect Behav. 8:781–799

Dickinson, J.L. (2001). Extrapair copulations in western bluebirds (*Saliamexicana*): female receptivity favors older males. Behav. Ecol. Sociobiol. 50:423-429.

Grant, B.R. and Grant, P. R. (1987). Mate choice in Darwin's finches. Biol. J. Lin. Sot. 32: 247-270.

Hansen, T.F. and Price D.K. (1995). Good genes and old age, do old mates provide superior genes? Evol. Biol. 8: 759-778.

Hegde, S.N. and Krishna M.S. (1997). Reproductive success of large and small flies in *Drosophila bipectinata* complex.Curr. Sci., 72: 747-750.

Jones, T.M.; Balmford, A. and Quinnell R.J. (2000). Adaptive female choice for middle-aged mates in a leaking Sandfly. Proc. R. Soc. Lond., 267: 681-686.

Kokko, H. (1997). Evolutionary stable strategies of age dependent sexual advertisement. Behav. Ecol. Sociobiol. 41:99-107.

Kokko, H. and Lindstrom, J. (1996). Evolution of female preference for old males. Proceedings of the Royal Society of London., 263:1533-1538.

Kruger, O. 2005.Age at first breeding and fitness in goshawk *Accipiter gentilis*. J.Anim Ecol. 74:266–273.

Manning, A.(1961). The effects of artificial selection for mating speed in *Drosophila melanogaster*. Animal Behaviour., 9: 82-92.

Moore, P.J. and Moore A.J. (2001). Reproductive aging and mating: the ticking of the biological clock in female cockroaches. ProcNatlAcadSci USA. 98:9171–9176.

Leips, J. and Mackay, T.F.C. (2000). Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic back-ground and larval density. Genetics., 155:1773-1788.

Lubkinbill, L, R Arking, MJ Clare, WC Cirocco, Buck, S. (1984). Selection of delayed senescence in *Drosophila melanogaster*. Evolution 38:996-1003.

Maklakov, A.A., Kremer, N.,G. Arnqvist. (2007). The effects of age at mating on female life-history traits in a seed beetle. Behav Ecol. 18:551–555.

Martin, J., Horwich, A.L., Hartl, F.U. (1992). Prevention of protein denaturation under heat stress by the Chaperonin Hsp60. Science. 58: 995-998.

Nuzhdin, S. V., E. G. Pasyukova, C. L. Dilda, Z.-B.Zeng, and Mackay, T. F. C. (1997). Sex-specific quantitative trait loci affecting longevity in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 94:9734-9739.

Omkar, Singh, SK., Singh, K. (2006). Effect of age on reproductive attributes of an aphidophagus ladybird, *Cheilomenessexmaculata*. Insect Sci. 13:301–308.

Partridge, L. & Barton, N.H. (1996). On measuring the rate of ageing. Proceedings of the Royal Society of London: Series B, 263:1365–1371

Partridge, L. and Fowler, K. (1992). "Direct and correlated responses to selectionon age at reproduction in *Drosophila melanogaster*."Evolution. 46: 76-91.

Pervez, A.; Omkar., Richmond, A.S. (2004). The influence of age on reproductive performance of the predatory ladybird beetle, *Propyleadissecta*. J. Insect Sci. 4:22.

Proulx, S.R., Day., Rowe, T.L. (2002). Older males signal more reliably. Proc R SocLond B. 269:2291–2299.

Promislow, D. E. L., M. Tatar, A. A. Khazaeli, and Curtsinger, J.W. (1996). Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. Genetics. 143:839-848.

Rose, M. R. (1984). Laboratory evolution of postponed senescence in *Drosophila melunoguster*. Evolution. 38: 1004- 1010.

Rose, M. R. and Charlesworth, B. (1980). A test of evolutionary theories of senescence.Nature. 287:141-142.

SPSS I, (1999). SPSS for Windows, 10.0.1.SPSS.

Spiess E.B. (1987). Discrimination among prospective mates in *Drosophila*. In: Fletcher DJC and Michener C.D (eds) Kin Recognition in Animals. John Wiley & Sons Ltd, New York, p 75-119.

Spieth HT, Ringo, J.M. (1983). Mating behavior and sexual isolation in *Drosophila*. InM Ashburner, ML Carson, JN.

Torres-Vila, L.M., Rodriguez-Molina, M.C. and Stockel, J. (2002). Delayed mating reduces reproductive output of female European grapevine moth, Lobesiabotrana (Lepidoptera: Tortricidae). B Entomol Res. 92:241–249.

Weatherhead, P.J. and Boag, P.T. (1995). Pair and extra-pair mating success relative to male quality in red-winged blackbirds. Behav. Ecol. Sociobiol. 37: 81–91.

Wolfner M.F. (1997). Tokens of love: functions and regulation of *Drosophila* male accessory gland products. Insect BiochemMol Biol. 27:179–192.

Wenninger, E.J., Averill, A.L. (2006). Effects of delayed mating on reproductive output of female oriental beetle *Anomalaorientalis* (Coleoptera: Scarabaeidae). Agric For Entomol. 8:221–231.

Zwaan, B., R. Bijlsma, and Hoekstra, R. E. (1995). Direct selection on life span in *Drosophila melanogaster*.Evolution. 49:649-659.