EFFECT OF FLUOXETINE ON THE COURTSHIP LATENCY, MATING LATENCY AND COPULATION DURATION OF DROSOPHILA MELANOGASTER

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ABSTRACT

Objective: *To study the effects of fluoxetinean antidepressant drug on courtship behaviour of Drosophila melanogaster.*

Material and Methods: *Larval and adult feeding methods were used for administration of the drug. LC50 of fluoxetine for these two protocols were estimated, then, three sub lethal concentrations (0.02, 0.04 and 0.06% for larval feeding and 0.4, 0.8 and 1.6% of fluoxetine for adult feeding method) were administered. Following combinations of crosses (control, male, female and both treated groups) in parents were made for observing the effect of antidepressant drug on the courtship behavioural elements such as, courtship latency, matting latency and copulation duration. The F1 and F2 progeny obtained from inbreeding of the above combinations were also used for observation.*

Results: *Courtship latency, matting latency and copulation duration were increased in all treated batches of parents for both larval and adult feeding methods. In larval feeding for treated batches, ANOVA (F value) of Courtship Latency was 19.055 & 1.863; of Mating latency was 0.644 & 0.174 and Copulation Duration was 1.145 & 7.135 for parents and F1 generation respectively.In Adult feeding for treated batches, ANOVA (F value) of Courtship Latency was 4.804 & 18.593; Mating Latency was 0.353 & 8.459 and Copulation Duration was 5.362 & 0.711 for parents and F1 generation respectively.*

Conclusion: *The courtship behaviours are affected by treatment of Fluoxetine; however the drug has no effect on courtship in most of groups and treatments of F1 and F2 progeny.*

Key words: *Fluoxetine, Antidepressant drugs, Courtship Behaviour, Drosophila Melanogaster.*

INTRODUCTION

Fluoxetine is an antidepressant drug belonging to Selective Serotonin Reuptake Inhibitors (SSRI) group. It is used to treat depression, obsessive-compulsive disorders, and bulimia (binge eating and purging). Although some studies that are the prerequisites of drug testing has been made, its effect on sexual behaviour of any species has not been studied. The fruit fly *Drosophila melanogaster* is a particularly suitable model for studying signals involved in the success of courtship. In this Dipteran's species, courtship consists of sequential stereotyped elements of behaviour that are primarily under genetic control[1,2]. Courtship pattern of different species has been studied by Bastock and Manning, Spieth, Cowling, Willmund and Ewing[3-7]. During courtship males and females exchange various types of acoustic, visual, chemical and tactile signals that culminate in copulation[8]. Such signals are also species specific and carry information about species, gender, and receptivity, and are used to modulate the responses of either male or female. This behaviour not only is influenced by the mutual stimuli and response generated by courting individuals but also by the environmental factors. In the present studies the effect of antidepressant drug fluoxetine on courtship behaviour *Drosophila* has been studied.

MATERIAL AND METHODS

The effect of fluoxetine was studied by larval and adult feeding methods. Wheat cream agar medium containing sub lethal concentrations of fluoxetine (0.02, 0.04 and 0.06% for larval feeding and 0.4, 0.8 and 1.6% for adult feeding method) was prepared and distributed to food vials[9]. In larval feeding technique, newly hatched

larvae were continuously fed on the above food medium. When adults emerged, virgin females and bachelor males were isolated within 3 hours of eclosion and maintained separately in normal media for 5 days for observation of the courtship behaviour. In adult feeding technique, virgin females and bachelor males emerged from the normal media were isolated and maintained them separately in normal media for 3 days, then transferred to treated media of different concentrations and fed for 2 days (48 hours). Thus they were aged for 5 days. These flies were used to study the effect of fluoxetine through larval and adult feeding experiments. The normal medium was used as control.

Following combination of crosses in treated parents were made for observing the effect of antidepressant drug on courtship behaviour.

(i) Control cross (Untreated ? x Untreated ?).

(ii) Male treated crosses (Treated ? x Untreated ?).

(iii) Female treated crosses (Treated? x Untreated ?).

(iv) Both treated crosses (Treated ? x Treated ?).

After observation of the behavioural parameters listed above, the pairs were kept in individual vials with normal media to obtain the progeny. When progeny appeared the virgins and bachelors were collected from each group and aged for 5 days. One male and one female from each group were again crossed together and their behaviour was observed. These crosses were comparable to the F1 inbreeding. This procedure is intended to know the long-term effect of these antidepressant drugs. Subsequently the F2 progeny was also obtained for each of the above crosses and their courtship behaviour were observed.

For observing sexual behaviour of *D. melanogaster* in control and different crosses of treated groups, a single virgin female was aspirated out gently and introduced into mating chamber (Rectangular glass boxes of 55x55x20 mm with a glass lid). A bachelor male was added to it and allowed to acclimatize to the chamber for 30 seconds, and then courtship and mating were directly observed. Continuous observations were made for 60 minutes, and then if there had been no copulation, the pair was replaced by a fresh pair. Courtship latency (time between introduction of male and female together into mating chamber until orientation of male towards female), mating latency (time between introduction of male and female together into mating chamber until initiation of copulation of each pair) and

EFFECT OF FLUOXETINE ON COURTSHIP LATENCY IN D. MELANOGASTER

		Concentration (%)	Parents	F1 Generation	F2 Generation
Larval feeding	Control		1.70±0.42	0.90±0.18	1.20±0.20
	Male treated	0.02	2.00±0.21	1.30±0.21	1.00±0.21
		0.04	3.30±0.67	1.40±0.27	0.90±0.23
		0.06	5.00±0.65	1.20±0.25	1.20±0.13
	Female treated	0.02	1.10±0.23	0.60±0.16	1.00±0.21
		0.04	2.80±0.42	1.80±0.29	1.00±0.21
		0.06	2.60±0.40	1.10±0.18	0.90±0.23
	Both treated	0.02	1.70±0.26	1.50±0.17	1.20±0.25
		0.04	3.10±0.57	1.20±0.20	1.00±0.25
		0.06	4.30±0.62	1.40±0.22	0.70±0.21
Adult feeding	Control		1.25±0.24	1.30±0.15	1.50±0.43
	Male treated	0.4	1.95±0.25	1.60±0.37	1.30±0.30
		0.8	2.30±0.33	2.60±0.31	1.30±0.30
		1.6	2.75±0.28	1.70±0.21	1.70±0.30
	Female treated	0.4	1.30±0.19	1.40±0.37	1.30±0.26
		0.8	1.35±0.13	3.20±0.25	1.50±0.17
		1.6	1.75±0.20	1.30±0.15	1.30±0.30
	Both treated	0.4	1.65±0.20	1.30±0.15	1.40±0.27
		0.8	1.75±0.18	1.80±0.25	1.40±0.16
		1.6	2.10±0.24	1.20±0.25	1.10±0.23

Values represent mean duration in minutes and their standard errors.

Table 1

EFFECT OF FLUOXETINE ON MATING LATENCY IN D. MELANOGASTER

		Concentration (%)	Parents	F1 Generation	F2 Generation
Larval feeding	Control		12.40±1.60	10.30±3.08	8.70±1.73
	Male treated	0.02	16.00±2.80	9.30±1.33	10.10±2.06
		0.04	16.50±6.34	7.40±1.38	8.40±0.98
		0.06	14.80±2.43	9.00±1.06	10.10±1.87
	Female treated	0.02	15.80±2.73	8.30±1.58	8.80±1.39
		0.04	12.20±4.25	12.50±1.96	7.40±1.40
		0.06	9.90±2.91	12.20±1.04	8.50±1.61
	Both treated	0.02	19.10±2.64	11.80±2.98	7.90±1.72
		0.04	13.50±3.50	10.80±2.33	7.90±1.28
		0.06	18.00±3.66	11.30±3.21	8.80±1.94
Adult feeding	Control		10.50±1.69	11.20±1.40	8.90±2.43
	Male treated	0.4	15.35±2.15	9.20±2.48	11.00±1.69
		0.8	10.90±1.28	21.10±2.85	8.10±1.91
		1.6	11.50±2.60	5.80±1.94	5.50±1.54
	Female treated	0.4	13.50±3.33	2.80±0.47	13.00±2.01
		0.8	10.55±1.97	12.00±2.09	9.50±2.13
		1.6	10.30±2.09	7.40±1.80	10.30±1.85
	Both treated	0.4	16.45±6.14	11.30±3.24	10.60±2.10
		0.8	17.90±4.23	7.90±1.64	6.40±2.23
		1.6	21.93±3.11	8.30±2.18	6.90±1.85

Values represent mean duration in minutes and their standard errors.

Table 2

copulation duration (time between initiation of copulation to termination of copulation of each pair) were recorded. 10 pairs were observed for each cross. All experiments were made during morning (7-11 A.M.) in a room with a temperature of 24±2º C under normal laboratory light condition.

Statistical analysis: For the purpose of statistical analysis of the effect of antidepressant drug the above combinations were divided into four groups and four treatments in the treated parents, F1 and F2 progeny. The groups included, control, male treated, female treated and both treated; while the treatments included control, first, second and third concentrations.

The data were compiled; means and standard errors were calculated. Two-way ANOVA and the Post Hoc Test of DMRT (Duncan's Multiple Range Test) were also carried out for each of the parameters analyzed by using SPSS software version 10.

RESULTS

Mean courtship latency in treated parents with fluoxetine was increased in all concentrations when compared to controls for both the larval and adult feeding methods, except in lowest concentration (0.02%) of female treated group in larval feeding method (Table 1). The longest courtship latency in parents was in the highest concentration of each treated groups. Mean courtship latency in control group of larval feeding was 1.70±0.42 minutes, while among treated batches, longest courtship latency was noticed in 0.06% concentration of male treated (5.00±0.65 minutes) group. In adults fed with fluoxetine, courtship latency in control was 1.25±0.24, while in 1.6% concentration of male treated group it was 2.75±0.28 minutes. ANOVA shows that the difference in mean courtship latency ($P<0.05$) between groups and between treatments of both larval and adult feeding (Table 4) was significant. The present data thus indicates that fluoxetine has brought out a decrease in the vigor of treated male. The F values obtained for courtship latency of F1 progeny of adult fed batches showed significant differences between both groups and treatments. Further, no variation of courtship latency was noticed in F2 progeny in both larval and adult feeding methods.

Mean mating latency in parents of larval and adult feeding technique were more than their respective controls, except in 0.04 and 0.06% of female treated in larval feeding and 1.6% concentration of female treated group in adult

EFFECT OF FLUOXETINE ON COPULATION DURATION IN D. MELANOGASTER

		Concentration (%)	Parents	F1 Generation	F2 Generation
Larval feeding	Control		17.90±0.75	23.80±1.03	23.30±1.08
	Male treated	0.02	18.90±0.57	22.30±0.79	22.50±0.86
		0.04	21.50±0.99	22.70±0.99	23.60±0.40
		0.06	19.80±0.92	21.10±0.48	23.10±0.78
	Female treated	0.02	20.60±1.22	24.50±1.04	20.80±0.63
		0.04	20.60±1.15	21.40±0.60	25.10±1.04
		0.06	17.40±0.58	21.40±0.95	25.40±1.03
	Both treated	0.02	17.80±0.94	25.10±0.97	22.00±0.77
		0.04	17.60±0.75	22.50±0.96	22.30±0.73
		0.06	19.20±1.08	21.20±1.00	22.80±0.85
Adult feeding	Control		24.60±0.69	23.50±1.07	24.10±0.71
	Male treated	0.4	20.75±0.91	23.50±0.92	24.60±1.19
		0.8	24.75±0.82	24.90±1.17	23.10±1.19
		1.6	26.45±0.76	22.80±1.04	24.60±1.11
	Female treated	0.4	25.15±0.57	25.80±0.94	25.50±1.07
		0.8	25.80±0.79	25.70±1.17	22.80±0.65
		1.6	25.05±0.84	28.10±0.82	26.10±1.07
	Both treated	0.4	23.40±0.66	23.30±1.39	23.50±1.13
		0.8	23.60±0.77	25.10±1.26	25.90±1.07
		1.6	23.35±0.68	24.20±1.33	26.30±1.12

Values represent mean duration in minutes and their standard errors.

Table 3

feeding method (Table 2). F values calculated for mating latency in adult feeding method showed significant variation ($P<0.05$, Table 4), among groups of parents, and groups and treatments of F1 progeny.

Although there was sharp increase in mating latency in batches treated with fluoxetine in the parent generations in larval feeding methods, most of treated groups and concentrations of fluoxetine were statistically insignificant, indicating that this antidepressant drug don't affect mating latency. The fact that the courtship latency is significantly increased in all concentrations, which is indicator of the decrease in vigour of males, and mating latency not significantly, suggests that the antidepressant drug has not affected the receptivity of females. The mating latency of both F1 and F2 progeny obtained from treated parents was insignificant.

Copulation duration was longest (26.45±0.76 minutes) in 1.6% concentration of male treated group of adult feeding. In larval feeding method copulation duration was longest (21.50±0.99 minutes) in 0.04% concentration of male treated group (Table 3). Table 4 shows significant differences in most of groups and

ANOVA (F VALUES) FOR COURTSHIP LATENCY, MATING LATENCY AND COPULATION DURATION OF D. MELANOGASTER FED WITH FLUOXETINE

		Courtship latency		Mating latency		Copulation duration	
		Parents	F1	Parents	F1	Parents	F1
Larval feeding	Group	5.598*	0.660	1.177	1.453	3.273*	0.755
	Treatment	19.055*	1.863	0.644	0.174	1.145	7.135*
Adult feeding	Group	10.721*	4.238*	5.983*	3.584*	4.978*	5.327*
	Treatment	4.804*	18.593*	0.353	8.459*	5.362*	0.711

** Mean difference is significant at 0.05 level by two way ANOVA*

Table 4

treatments, with copulation duration higher than their respective controls. ANOVA of this data in larval feeding shows that the values were significant (P<0.05) between groups of parents, and among treatments of F1 progeny (Table 4). ANOVA calculated for copulation duration of adult treated groups show that groups and treatments of parents and also groups of F1 progeny were significant. From the post hoc test of DMRT it is evident that male and both treated batches were significantly affected in larval feeding.

DISCUSSION

In *Drosophila*, successful mating depends on male's activity and female's, receptivity. Courtship latency is one of the parameters, which indicate vigor of male *D. melanogaster*[10]. It represents the time between introduction of male and female flies into observation chamber and initiation of courtship[11-13]. A male with high vigor reacts quickly in the presence of female while a male with less vigor, reacts slowly[14].

In the present studies, courtship latency is affected by the treatment of fluoxetine. Courtship latency has increased in all treated batches of parents for both larval and adult feeding methods, except in 0.02% concentration of female treated with fluoxetine. The values were significantly different (P<0.05, by ANOVA) in most flies of both groups and treatments. Increase in courtship latency means decrease in the vigor of males. The present data thus indicates that the drug has brought out a decrease in the vigor of treated male. Fluoxetine have no effect on courtship latency in most of groups and treatments of F1 and F2 progeny. This indicates that this antidepressant drug affect immediately after treatment or only in first generation. This result agrees with the finding of Nagabhushana, while studying the effect of Dithane M-45 on *D. Melanogaster*[15].

Mating latency indicates both vigor of males and receptivity of females. It is the time required for males and females to initiate copulation. Higher the vigor of males and receptivity of females, shorter is the mating latency. During this period, courtship acts are performed mostly by males, to increase receptivity of females and to make her sexually excited[16]. A male with high vigor has to perform same courtship act, more number of times to a non-receptive female than to a receptive female.

Although there is no study, which shows the effect of drugs or other chemicals on vigour of males and receptivity of females, studies of Santos *et al.* (1988), and Ruiz and Santos (1989), have demonstrated that body size influences mating latency[17,18]. Nagabhushana (2002) has reported that Dithane M-45 a pesticide increase the mating latency of *D. melanogaster*[15]. The mating latency of both F1 and F2 progeny in larval feeding method obtained from treated parents was insignificant. This indicated that the change in the mating latency is only temporary and not carried over to subsequent generations.

Courtship is a prerequisite for copulation in *D. melanogaster*[19]. Copulation duration is the time between initiation of copulation to termination of copulation of each pair. It is quite natural that copulation is severely affected when courtship is affected. Perusal of table 3 shows that copulation duration of *D. melanogaster* in larval and adult feeding methods was affected by different concentrations of fluoxetine. However copulation duration remains unaffected in female treated flies. The reason is obvious that fluoxetine severely affect male behaviour, thus affecting copulation duration. This result agrees with the finding of Nagabhushana, where he observed increased copulation duration of male treated with Dithane M-45[15]. The ANOVA and DMRT also showed that fluoxetine has no effect on copulation duration of *D. melanogaster* in F2 progeny.

CONCLUSION

Thus the results of these experiments show that fluoxetine affects the sexual behavior of males and not females. Further the effect is temporary and not carried over to subsequent generations.

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REFERENCES

1. Hall JC. The mating of a fly. Science 1994; 264: 1702-14.
2. Yamamoto D, Jallon JM, Komatsu A. Genetic dissection of sexual behaviour in Drosophila melanogaster. Ann Rev Entomol 1997; 42: 551-85.
3. Bastock M, Manning A. The courtship of Drosophila melanogaster. Behaviour 1955; 8: 86-111.
4. Spieth HT. Courtship behavior in Drosophila. Ann Rev Entomol 1974; 19: 385-405.
5. Speith HT. Courtship patterns and evolution of the Drosophila adiastola and Plantibia species subgroups. Evolution 1978; 32: 435-51.
6. Cowling DE. The genetics of Drosophila melanogaster courtship song - diallel analysis. Heredity 1980; 45: 401-3.
7. Willmund R, Ewing A. Visual signals in the

courtship of Drosophila melanogaster. Anim Behav 1982; 30: 209-15.

8. Spieth HT, Ringo JM. Mating behavior and sexual isolation in Drosophila. In: Ashburner M, Carson HI,Thompson, Jr. J.N The Genetics and biology of Drosophila. Vol. 3c, Academic press, London 1983; 223-84.
9. Nazari M, Hegde SN. Toxic effect of flouxetine-an antidepressant drug on the rate of development and viability of *Drosophila melanogaster*. Dros Inf Serv 2003; 86: 55-7.
10. Eastwood L, Burnet B. Courtship latency in male D. melanogaster. Behav Genet 1977; 7: 359-72.
11. Markow TA. A comparative investigation of the mating system of D. hydei. Anim Behav 1985; 33: 775-81.
12. Markow TA. Reproductive behaviour of D. melanogaster and D. nigrospiracula in the field and in the laboratory. J Comp Psychol 1988;102: 169-73.
13. Hegde SN, Krishna MS. Size assortative mating in Drosophila malerkotliana. Anim Behav 1997; 44: 419-26.
14. Markow TA. Male mating experience and competitive courtship success. In: D. melanogaster. Nature Lond 1978; 276: 821-2.
15. Nagabhushana. Studies on the biodiversity of Drosophilids in Devarayanadurga Karnataka, India. Ph.D. thesis of Kuvempu University, Karnataka- India, 2002.
16. Spieth HT. Evolutionary implications of sexual bahaviour in Drosophila. In: Evolutionary biology (Dobhansky T, Hecht MK and Steer WC) Appleto- Century- Crofts, New York, 1968: 157-93.
17. Santos M, Ruiz A, Barbadilla A. The evolutionary history of D. buzzatti XIV. Largerflies mate more pften in nature. Heredity 1988; 61: 255-62.
18. Ruiz A, Santos M. Mating probability, body size and inversion polymorphism in a colonizing population of *D. buzzetti*. In: Evolutionary Biology of Transient Unstable Populations. Ed. Fontdevila, A. Springer-Verlag. Berlin 1989; 96-113.
19. Spiess EB. Mating propensity and its genetic basis. In: Drosophila Essays in Evolution and Genetics in Honour of T. Dobzhansky (Hecht M.K and Steere, WC) North Holland, Amesterdam, 1970: 315-79.

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