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RESEARCH ARTICLE

FRUCTOSE INDUCED VARIATIONS IN PUPATION HEIGHT IN *BIPECTINATA* SPECIES COMPLEX OF GENUS- *DROSOPHILA*

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ABSTRACT

To study the pupation site preference which is an important larval behavior, pupation height was scored in Drosophila malarkotliana, D.bipectinata and D. parabipectinata using fructose sugar in culture media. Strains of these species were collected from Unchahar, Bachhrawan, Raebareli & Salon localities in Raebareli Districts. The mean pupation height in different strains of Drosophila malarkotliana ranged from 3.56 to 3.67, in D. bipectinata ranged from 4.58 to 5.02 and D.parabipectinata., ranged from 1.96 to 2.03. The analysis of variance and t-test were performed to test intra and interspecies in pupation height. These tests revealed significant variation among different strains of the species. Significant variations among male & female for pupation height the same species were also found in all species Drosophila. These observations provide evidence for ecogenetics variations in pupation height of three species of Drosophila. Variations among different strains of the same species in pupation height are attributable to genetic heterogeneity among the strains.

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INTRODUCTION

Research in field of behavior genetics largely began as a byproduct of other investigations in a number of organisms including *Drosophila*. Out of four life stages of *Drosophila* two stages exhibit behavior-larva and adult. Although sexual and nonsexual behavior of adult has been extensively studied (Grossfield, J., 1978; Chatterjee and Singh., 1989, Spieth and Ringo., 1983; Gupta and Kumar., 2017; Gupta *et al.*, 2018; Ramniwas and Kumar., 2019) the behavior of larvae has been largely ignored until recently. In view of the fact that the total fitness is heavily influenced at the larval stage, behavior genetic study of *Drosophila* larvae has been initiated recently. The pupation site preference is an important step in *Drosophila* preadult development because the place selected by larvae can have a decisive influence on their subsequent survival (Sameoto and Miller 1968). Thus pupation site preference is interesting and important primarily because it affects the survival of pupae. In *Drosophila*, the choice of a suitable pupation site directly influences the successful emergency of the adult (Sokolowlski, 1985; Rodriguez *et al*,, 1992). Previous studies show that differences in pupation height, a continuous measure of pupation site preferences, are influenced by the abiotic factors moisture, lighting condition, and temperature and by the biotic factors density, sex developmental time, and species measured (for reviews, see Sokolowski, 1985; Sokolowski *et al.*, 1986; Vandal *et al.*, 2010; Nakul *et al*., 2008; Deniz and David 2013; Narasimaha *et al*., 2015), Pupation site choice by *Drosophila* larvae could provide a basis for larval habital choice and niche separation between species. Schnebel and Grossfield (1986) have done the only systematic study of interspecicfic variability in pupation behavior in *Drosophilla*. They found significant differences between the two most closely related species in four species triads, each triads, and each triad coming from a different ecosystem ranging from desert to tropical rain forest. Earlier, Markow (1979) observed that *D. melanogaster* pupated higher than its sibling, *D. simulans*, and that *D. pseudoobscura*, a more distantly related species, pupated higher that *D. simulans* but lower than *D. melanogaster*. This, and Schnebel and Grossfield's (1986) findings, strongly suggests the presence of niche separation among closely related Drosophila larvae. The phylogenetic relationship among the members of the *D. melanogaster* subgroup has recently been considered in detail using genetic information from all available sources (Lachaise *et al.*, 1988; Singh, 1989). The present study is a systematic study of intersexual, intraspecific, and interspecific variability in pupation behavior and developmental time in the *Drosophila bipectinata* species complex of genus *Drosophila*.

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Three species of Drosophila viz, *Drosophila malarkotliana, D. bipectinata* and *D. parabiectinata* were used for present study using fructose sugar in culture media. Three species viz. *D. malerkotilana, D.bipectinata* and *D. parabipectinata* were used for strains of using fructose sugar in culture media.

MATERIALS AND METHODS

The handling and test procedures for measuring pupation height are described in detail by Bauer and Sokolowski (1985) and Pandey and Singh (1993).A large number of stocks of *D. malerkotliana, D. bipectinata* and *D. parabipectinata* maintained in our laboratory were employed during the course of the present study. In *D. malerkotliana*, three types of strains were used. (i) mass culture wild stocks (23) which were established from several naturally impregnated females collected from different localities in India, (ii) mutant stocks (7) and (iii) wild stocks which were made karyotypically homozygous for ST or inverted gene arrangements in different chromosomes and thus they are free of inversion heterozygosity. In *D.bipectinata*, 5 mass culture stocks of different geographic origin were utilized. Seven mass culture stocks of different geographic origin were used *D.malerkotliana*.

vial for mating and after 24 hr they were transferred to a petridish containing a thin layer of food medium for egg laying for about 48 hr. Then flies were discarded. After larval eclosion, 10 first instars larvae were removed and carefully seeded in fresh culture vial (25 mm diam × 100 mm length) and the vial was stoppered with cotton plug. For each strain, ten replicates were carried out. At the end of pupation time, the height of each pupa was measured. Pupation height was considered to be zero when larva pupated on food surface. These measurements were made when all the larvae had pupated but prior to the eclosion of adult. Pupation height was determined as the distance in mm of each pupa from the surface of food. In order to know the effect of sex on pupation height, each pupa after measurement of height was transferred to food vial and after the eclosion of adult its sex was noted. Thus mean pupation height was calculated for each strain on the basis of ten replicates (each replicate of 10 larvae).

RESULTS

Mean values of pupation height in mass culture wild stocks from two different population *of D.bipectinata* are given in (Table1a).

Table 1. Mean value of pupation height (mm) in Fructose sugar

1 (a). Mean value of Pupation height (mm) is different mass culture normal strain of *D. bipectinata*

Strain	Pupation height ± SE			Total no. of pupae scored	Sex			
					Male	N	Female	N
Unchahar	4.89	±	0.046	100	4.94	48	4.38	47
Raebareli	4.53	+	0.025	100	3.94	54	4.79	42
Mean	4.71		0.035207		4.44		4.58	
	t = 1.86, P>0.05							

1 (b). Mean value of Pupation height (mm) is different mass culture normal strain of *D. malerkotliana*

Strain	Pupation height ± SE			Total no. of pupae scored	Sex			
					Male	N	Female	N
Unchahar	3.56	±	0.03	100	4.57	44	3.2	52
Bachharawan	3.51	±	0.02	100	4.65	46	3.9	54
Raebaereli	3.57	±	0.02	100	4.36	47	3.0	53
Salon	3.58	±	0.02	100	4.12	51	4.1	49
Mean	3.555		0.02		4.42		3.5	
	F = 0.79, P<0.05							

1(c). Mean value of Pupation height (mm) is different mass culture normal strain of *D. parabipectinata*

Strain	Pupation height ± SE			Total no. of pupae scored	Sex			
					Male	N	Female	N
Unchahar	1.94	±	0.032	100	3.2	42.0	3.4	48
Bachharawan	1.9	±	0.028	100	2.9	47.0	3.2	44
Raebaereli	1.86	±	0.029	100	4.0	53.0	2.7	47
Salon	1.87	±	0.045	100	4.3	50.0	3.8	42
Mean	1.8925		0.033587		3.6		3.3	
	F = 0.78, P<0.05							

Table 2. Comparison of pupation heights between different species

S. no.	Species	t- value	df	P
1	*D. malerkotliana vs D. bipectinata*	-10.85	4	<0.05
2	*D. malerkotliana vs D. parabipectinata*	88.11	6	>>0.05
3	*D.bipectinata vs D. parabipectinata*	26.79	4	>0.05

All the strains were cultured on simple culture medium containing agar, dried yeast, fructose, maize powder, nipagin, propionic acid and water. All the experiments were conducted in a room maintained at approximately 24^0C temperature with 60-80% relative humidity and 12:12 hr light and dark cycle. Virgin female and males were collected and 5 days old flies were used to initiate the experiments. Ten females and 10 males were placed in food

The average pupation height is 4.71 mm with fluctuation between 4.53 to 4.89 mm. To test variation in different strains analysis of variance was performed. The difference among the strains is highly singinficant. The average pupation height for mass culture wild strocks from different papulation of *D.bipectinata* has been considered as mean pupation height for species. Mean values of pupation height in mass culture wild stocks from two different population of

D.malerkotliana are given in (Table1b). The average pupation height is 3.55 mm with fluctuation between 3.51 to 3.58 mm. To test variation in different strains analysis of variance was performed. The difference among the strains is highly significant. The average pupation height for mass culture wild strokes from different population of *D.bipectinata* has been considered as mean pupation height for species. Mean values of pupation height in mass culture wild stocks from four different population of *D.parabipectinata* are given in (Table1c). The average pupation height is 1.89 mm with fluctuation between 1.86 to 1.94 mm. To test variation in different strains analysis of variance was performed. The difference among the strains is highly significant. The average pupation height for mass culture wild strocks from different population of *D.bipectinata* has been considered as mean pupation height for species. A comparison of pupation height in male and female flies of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. bipectinata* for fructose sugar was 4.44 mm with flucuation between 3.94 to 4.94 mm. For femele larvae, the mean value of pupation height was 4.58mm slightly lower than male larvae and fluctuates between 4.38 to 4.79mm (Table1a). A comparison of pupation height in male and female flies of each strain of all the three species was also observed.

Table 3. Comparison of pupation heights between male and female

S. no.	Species	t- value	df	P
1	*D. malerkotliana*	4.36	6	>0.05
2	*D. parabipectinata*	0.77	6	<0.05
3	*D.bipectinata*	1	2	<0.05

The mean pupation height of male larvae of *D. parabipectinata* for fructose sugar was 3.6 mm with fluctuation between 2.9 to4.3 mm. For female larvae, the mean value of pupation height was 2.3mm slightly lower than male larvae and fluctuates between 2.7 to 3.8 mm (Table1b). A Comparison of pupation height in male and female flies of each strain of all the three species was also observed. The mean pupation height of male larvae of *D.malerkotliana* for fructose sugar was 4.42mm with fluctuation between 4.12 to4.65 mm. For female larvae, the mean value of pupation height was 3.5 mm slightly lower than male larvae and fluctuates between 3.0 to4.1 mm (Table1c). Comparison of pupation height between three different species of collected from different natural ecogeographic regions and cultured using fructose as resource media are given in Table (2). It is clear from t test value that all the three species differ significantly from each other. *D.bipectinata* has higher pupation height than *D.malerkotliana* and *D.parabipactinata* has lowest mean pupation height (Table 3).

DISCUSSION

In case of Fructose sugar Comparison of pupation height between three different species of collected from different natural ecogeographic regions and cultured using fructose as resource media are given in Table 2 and 3. It is clear from t test value that all the three species differ significantly from each other. *D. bipectinata* has higher pupation height than *D.malerkotliana* and *D. parabipactinata* has lowest mean pupation height. A comparison of pupation height in male and female flies of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. bipectinata* for fructose sugar was 4.44 mm with fluctuation between 3.94 to 4.94 mm. For female larvae, the mean value of pupation height was 4.58mm slightly lower than male larvae and fluctuates between 4.38 to 4.79mm. A comparison of pupation height in male and female flies of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. parabipectinata* for fructose sugar was 3.6 mm with fluctuation between 2.9 to4.3 mm. For female larvae, the mean value of pupation height was 2.3mm slightly lower than male larvae and fluctuates between 2.7 to 3.8mm. A Comparison of pupation height in male and female flies of each strain of all the three species was also observed. The mean pupation height of male larvae of *D.malerkotliana* for fructose sugar was 4.42mm with fluctuation between 4.12 to4.65 mm. For female larvae, the mean value of pupation height was 3.5 mm slightly lower than male larvae and fluctuates between 3.0 to4.1mm. Comparison of pupation height between male and female larvae of all the three species collected from different wild ecological region and mass cultured in lab. Condition using fructose sugar is given in table 1**.** It is obvious from t-test value that all the three species differs significantly for its male and female pupation height. D. *bipectenata* males have highest mean pupation height (4.44mm) than *D..malerkotliana* (4.42mm) and *D. parabipectinata* have lowest mean pupation height, in males (3.60mm). In female also, *D.bipectinata* has highest pupation height (4.58mm) followed by *D.malerkotliana* (3.5mm) and *D. parabipectinata* (3.3mm). These variations in pupaqtion height among difference species of *Drosophila* in Fructose sugar compound are attributable to genetic heterogeneity among the diets tested (Singh B.N, 1988). *Drosophila bipectinata* pupation heights is higher than *D. malerkotliana* and *Drosophila bipectinata* because of species variation and different geographic origin, significant variations among all three species were observed. There is extensive evidence for genetic differentiation in Indian population of *Drosophila* (Grossfield), 1978). *D. parabipectinata* shows very lowers pupation height compare to *D. malerkotliana* and *Drosophila bipectinata*. The analysis of food materials and there geographic distribution appears to have more genetic variation than *D. malerkotliana* and *Drosophila bipectinata* (Sokal R. R. 1960).

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