

Ecogenetic variations in pupation height in *bipectinata* species complex of genus-*Drosophila*

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Abstract

In order 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 sucrose 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 5.02and *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.

KEYWORDS: *Drosophila*, Sucrose, Pupation height. Selection, Ecogenetics.

1. Introduction

The study of behavior is relatively a new field of investigation and research in behavior genetics largely began as a byproduct of other investigations in a number of organisms including *Drosophila*. Two of the four life stages of *Drosophila* 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), 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 (Sokolowski, 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 habitat choice and niche

separation between species. Schnebel and Grossfield (1986) have done the only systematic study of interspecific variability in pupation behavior in *Drosophila*. 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 than *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*. Three species of *Drosophila* viz, *Drosophila malerkotliana*, *D. bipectinata* and *D. parabipectinata* were used for present study using sucrose sugar in culture media.

Three species viz. *D. malerkotliana*, *D. bipectinata* and *D. parabipectinata* were used for strains of using sucrose sugar in culture media.

2. 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*. All the strains were cultured on simple culture medium containing agar, dried yeast, sucrose, maize powder, nipagin, propionic acid and water. All the experiments were conducted in a room maintained at approximately 24°C 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 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).

3. Result

In the *D. bipectinata* the average pupation height was 4.8 mm with fluctuation between 4.58 to 5.02 mm. To test variation in different strains t-test was performed. The difference among the strains is highly significant ($t=10.6, P<0.05$). The average pupation height for mass culture wild stocks from different population of *D. bipectinata* has been considered as mean pupation height for species (**Table 1**).

The average pupation height of *D. malerkotliana* was 3.63 mm with fluctuation between 3.56 to 3.67 mm. To test variation in different strains analysis of variance was performed. The difference among the strains is highly significant ($F=13.76, P<0.05$). The average pupation height for mass culture wild stocks from different population of *D. bipectinata* has been considered as mean pupation height for species.

The average pupation height of *D. parabipectinata* is 1.99 mm with fluctuation between 1.96 to 2.03 mm. To test variation in different strains analysis of variance was performed. The difference among the strains is highly significant ($F=3.08, P<0.05$). The average pupation height for mass culture wild stocks from different population of *D. bipectinata* has been considered as mean pupation height for species.

Comparison of pupation height between three different species of collected from different natural ecogeographic regions and cultured using sucrose as resource media are given in **Table2**. 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.parabipectinata* has lowest mean pupation height (**Table3**).

Comparison of pupation height in male and female larvae of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. bipectinata* for sucrose sugar was 5.67mm with fluctuation between 5.38 to 5.96mm. For female larvae, the mean value of pupation height was 4.77mm slightly lower than male larvae and fluctuates between 4.73 to 4.82mm (**Table1a**).

A comparison of pupation height in male and female larvae of each strain of all the three species was also observed. The mean pupation height of male larvae of *D.malerkotliana* for sucrose sugar was 4.60mm with fluctuation between 4.16 to 5.19 mm. For female larvae, the mean value of pupation height was 4.09mm slightly lower than male larvae and fluctuates between 3.42 to 4.95 mm (**Table1b**).

A comparison of pupation height in male and female larvae of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. parabipectinata* for sucrose sugar was 3.62mm with fluctuation between 3.0 to 4.28 mm. For female larvae, the mean value of pupation height was 3.34mm slightly lower than male larvae and fluctuates between 2.76 to 3.93 mm (**Table1c**).

4. Discussion

In case of Sucrose sugar Comparison of pupation height between three different species of collected from different natural ecogeographic regions and cultured using sucrose 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. parabipectinata* has lowest mean pupation height.

A comparison of pupation height in male and female larvae of each strain of all the three species was also observed. The mean pupation height of male larvae of *D. bipectinata* for sucrose sugar was 5.67mm with fluctuation between 5.38 to 5.96mm. For female larvae, the mean value of pupation height was 4.77mm slightly lower than male larvae and fluctuates between 4.73 to 4.82mm. The mean pupation height of male larvae of *D. parabipectinata* for sucrose sugar was 3.62mm with fluctuation between 3.0 to 4.28 mm. For female larvae, the mean value of pupation height was 3.34mm slightly lower than male larvae and fluctuates between 2.76 to 3.93mm. where as the mean pupation height of male larvae of *D. malerkoliana* for sucrose sugar was 4.60mm with fluctuation between 4.16 to 5.19 mm. For female larvae, the mean value of pupation height was 4.09mm slightly lower than male larvae and fluctuates between 3.42 to 4.95mm.

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 sucrose sugar is given in **Table1**. It is obvious from t-test value that all the three species differs significantly for its male and female pupation height. *D. bipectinata* males have highest mean pupation height (5.67mm) than *D. malerkoliana* (4.60mm) and *D. parabipectinata* have lowest mean pupation height, in males (3.62mm). In female also, *D. bipectinata* has highest pupation height (4.77mm) followed by *D. malerkoliana* (4.09mm) and *D. parabipectinata* (3.34mm). These variations in pupation height among difference species of *Drosophila* in Sucrose sugar compound are attributable to genetic heterogeneity among the diets tested (Singh B.N, 1988). *Drosophila bipectinata* pupation height 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 et al, 1960).

Acknowledgement: The authors are grateful to the principal Feroze Gandhi College, Raebareli for providing necessary facilities.

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Table1: Mean value of pupation height (mm) in sucrose 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
Unchahar	5.02±0.028	100	5.38	47
Raebareli	4.58±0.013	100	5.96	52
Mean	4.8±0.02		5.67	4.77
				44

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
Unchahar	3.67±0.01	100	4.34	43
Bachharawa n	3.56±0.02	100	5.19	42
Raebareli	3.64±0.02	100	4.16	50
Salon	3.66±0.02	100	4.71	45
Mean	3.63±0.02		4.60	4.09
				41

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
Unchahar	2.03±0.014	100	3.20	40
Bachharawa n	1.99±0.009	100	3.00	46

Raebareli	1.97±0.020	100	4.00	51	2.76	49
Salon	1.96±0.025	100	4.28	50	3.93	42
Mean	1.99±0.02		3.62		3.34	

Table2: Comparison of pupation heights between different species.

S. no.	Species	t- value	df	P
1	<i>D. malerkotliana</i> vs <i>D. bipectinata</i>	-8.3	4	<0.05
2	<i>D. malerkotliana</i> vs <i>D. parabipectinata</i>	50.96	6	>>0.05
3	<i>D. bipectinata</i> vs <i>D. parabipectinata</i>	-20.52	4	>0.05

Table3: Comparison of pupation heights between male and female.

S. no.	Species	t- value	df	P
1	<i>D. malerkotliana</i>	1.28	5	<0.05
2	<i>D. parabipectinata</i>	0.71	6	<0.05
3	<i>D. bipectinata</i>	2.88	2	<0.05