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RESEARCH ARTICLE

LARVAL PUPATION SITE PREFERENCE IN *DROSOPHILA MELANOGASTER*, *D. SIMULANS, D. MAURITIANA* AND THEIR HYBRIDS

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ABSTRACT

Pupation site preference in *Drosophila melanogaster*, *D. simulans, D. mauritiana* and their hybrids were studied. The larvae of *D. melanogaster* prefer glass while its sibling species *D. simulans* and *D. mauritiana* prefers media to pupate. Progenies of direct and reciprocal cross of *D. melanogaster* and *D. simulans* preferred media for pupation. The hybrids of cross between *D. melanogaster* female and *D. mauritiana* male prefers glass for pupation. Their percentage of pupation is intermediate than the parents. Whereas, the hybrids of *D. simulans* female and *D. mauritiana* male prefer media for pupation and percentage of PSP are more than the parents.

INTRODUCTION

Drosophila life cycle consists of 4 stages, namely, egg, larva, pupa and adult. Larval period is an important stage in its life cycle, which moves to different surfaces for pupation. Pupation site preference (PSP) is an important event in *Drosophila* preadult development and involves habitat selection, because the place selected by larva can have decisive influence on their subsequent survival as pupae (Sameoto and Miller, 1968).

The PSP is one of the behaviour exhibited by late third instar larva in *Drosophila*. PSP has been analyzed by pupation height measurement and percentage of pupation site preference. It has been studied in different subgroups of *Drosophila* belonging to *melanogaster*, *virilis*, *repleta* and *immigrans* groups. The results revealed that, *immigrans* group preferred to pupate on media whereas *repleta* preferred to pupate on glass. *Drosophila melanogaster* group has been the focus of the most recent advances. In this group, *D. simulans* and *D. mauritiana* preferred media whereas, *D. melanogaster* preferred glass for pupation. Among the species analyzed, many species preferred media maximum for pupation and few species preferred glass. *D. rajasekari* and *D. gibberosa* preferred cotton for their pupation (Shirk *et al.,* 1988; Shivanna *et al.,* 1996; vandal *et al.,* 2003). The genetic study of pupation revealed that the differences between larvae that prefer to pupate on food and larvae that prefer bottom of the cages is simple and due mainly to a single gene difference (de Souza *et al.,* 1970). Behaviors related with habitat selection may be of great importance in determining the genetic structure of populations (Taylor, 1976).

The effect of abiotic and biotic factors on larval pupation height has been studied in different species and strains of *Drosophila* (Manning and Markow, 1981; Ringo and Wood, 1983; Sokolowski and Hanshell, 1983; Bauer and Sokolowski, 1985; 1988; Casares and Carracedo, 1986b; Sokolowski and Bauer, 1989; Singh and Pandey, 1993a & b). The genetic analysis showed that the second and third chromosomes act additively on pupation distance and third pair of chromosome have larger effect than the second (Bauer and Sokolowski, 1985; 1988; Casares and Carracedo, 1986b; Sokolowski and Bauer, 1989). The inheritance of intra and interspecies pupation behaviour is under the polygenic control (Singh and Pandey, 1993a & b). Genetic variability for pupation behaviour may be maintained through habitat selection in heterogenous environment (Rodriguez *et al.,* 1992).

Crosses between *D. melanogaster*, *D. simulans* and *D. mauritiana* have been made to study various aspects like hybrid sterility, lethality, gene expression, longevity, developmental stability etc. (Sawamura *et al.,* 1993; Carracedo *et al.,* 1998; Barbash and Ashburner, 2003; Ranz *et al.,* 2004; Bhadra *et al.,* 2006; Stamenkovic-Radaki *et al.,* 2009). Whereas, the PSP have not been studied in hybrids. *D. simulans* and *D. mauritiana* prefers media and *D. melanogaster* prefers glass for pupation (Shivanna *et al.,* 1996; vandal *et al.,* 2003). These species were taken to study the PSP in hybrids. Because, information about the genetic analysis of pupation site preference in hybrids is not available.

MATERIALS AND METHODS

D. melanogaster, *D. simulans* and *D. mauritiana* were collected from *Drosophila* stock center, Dept. of Zoology, University of Mysore, Mysore and cultured in wheat cream agar medium prepared as per the procedure described by Shivanna *et al.,* (1996). Yeast was added to cultures everyday to feed the larvae and to maintain moisture.

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Crossing experiment

The virgin male and female flies were collected within one hour of their eclosion from pupae and they were kept in separate vials. After 3 days, they were used for direct and reciprocal cross experiment as below.

D. melanogaster female X *D. simulans* male ⟶ F_1A
D. melanogaster male X *D. simulans* female ⟶ F_1B
D. melanogaster female X *D. mauritiana* male ⟶ F_1C
D. melanogaster male X *D. mauritiana* female ⟶ F_1D (No Progeny)
D. simulans female X *D. mauritiana* male ⟶ F_1E
D. simulans male X *D. mauritiana* female ⟶ F_1F (No Progeny)

Larval Pupation Site Preference

Synchronized first instar larvae about 50 were collected from each species and hybrids. Larvae were placed in different culture vials containing equal quantity of wheat cream agar medium to study the larval PSP. Ten replicates were maintained at a constant temperature of 22 ± 1°C with a relative humidity (RH) of 70 - 80 %. Extra yeast was added everyday to feed the larvae. The PSP has been studied using the procedure described by Shivanna *et al.,* (1996). The number of larvae pupated at three different sites (cotton, glass wall and media of culture vials) were counted and tabulated.

Statistical Analysis

Mean ± SD and percentage of pupation were calculated based on the larvae pupated at 3 different sites. The data was subjected to statistical analysis (using SPSS 13.0 software). Paired t-test was used to analyze the difference between sites and species, One-way ANOVA was done to know the difference between species at each sites and independent t-test has been carried out to know the difference between pure species and hybrids.

RESULTS

Mean ± SD and percentage value of PSP in *D. melanogaster*, *D. simulans* and *D. mauritiana* are given in table 1. The larvae of *D. melanogaster* prefer to pupate more on glass (94.2%) and less on media (2.6%), whereas *D. simulans* and *D. mauritiana* shows contrast results with *D. melanogaster*. i.e. they prefer to pupate less on glass (2.8% and 2.2%) and more on media (91.8% and 89.2%) respectively. None of the species preferred cotton for pupation. Mortality rate of *D. melanogaster*, *D. simulans* and *D. mauritiana* are 3.2%, 5.4% and 8.6% respectively. The difference between 3 species at glass and media are significant.

Table 2 shows the Mean ± SD and percentage values of PSP in hybrids. The larval progeny from *D. melanogaster* females and *D. simulans* males (F_1A) and *D. melanogaster* males and *D. simulans* females (F_1B progeny is less in number) show intermediate character of parental species. They prefer to pupate more on media (51.4% and 3.4%) and less on glass (36.0% and 1.0%) respectively. The difference between glass and media pupation is less in hybrids than parents. The larval progeny of *D. melanogaster* females and *D. mauritiana* males (F_1C) preferred glass 59.8%, media 27.0% and percentage of pupation is intermediate of parents. Progenies from *D. simulans* females and *D. mauritiana* males (F_1E) shows maximum media pupation (94.0%) and percentage of pupation is more than parents, whereas the glass pupation is very less (1.4%).

Table 2 Mean ± SD and percentage of pupation site preference of hybrids

	Glass	%	Media	%	Cotton	%
F_1A	18.0 ± 11.5	36.0	25.9 ± 9.3	51.4	0.0	0.0
F_1B	0.5 ± 0.85	1.0	1.7 ± 4.7	3.4	0.0	0.0
F_1C	29.9 ± 15.2	59.8	13.5 ± 15.5	27.0	0.0	0.0
F_1D	-	-	-	-	-	-
F_1E	0.7 ± 0.8	1.4	47.0 ± 1.3	94.0	0.0	0.0
F_1F	-	-	-	-	-	-

F_1A = *D. melanogaster* ♀ X *D. simulans* ♂
F_1B = *D. melanogaster* ♂ X *D. simulans* ♀
F_1C = *D. melanogaster* ♀ X *D. mauritiana* ♂
F_1D = *D. melanogaster* ♂ X *D. mauritiana* ♀
F_1E = *D. simulans* ♀ X *D. mauritiana* ♂
F_1F = *D. simulans* ♂ X *D. mauritiana* ♀

The PSP between *D. melanogaster*, *D. simulans* and *D. mauritiana* shows significant variations at both the sites (glass and media) except *D. simulans* and *D. mauritiana* at glass. The pupation of *D. melanogaster* shows significant difference with F_1A, F_1B and F_1C at both glass and media except at media of F_1B. *D. simulans* pupation difference is found to be significant with F_1A and F_1B at both the sites whereas with F_1E at media only. The pupation variation of *D. mauritiana* is highly significant with F_1C at both the sites and F_1E at media only.

Table 1 Mean ± SD and percentage of pupation site preference of pure lines

	Glass	%	Media	%	Cotton	%
D. melanogaster	47.1±4.35	94.2	1.3±2.83	2.6	0.0	0.0
D. simulans	1.4±0.97	2.8	45.9±0.99	91.8	0.0	0.0
D. mauritiana	1.1±0.99	2.2	44.6±1.26	89.2	0.0	0.0
ANOVA	1005.346*			1823.462*		-

df = 2 , 27 * $P < 0.001$ level.

Pure species and F_1E are significant at Glass v/s Media, the PSP of all pure species and hybrids are significantly differ at Glass v/s Cotton except F_1B and F_1E. The larvae of pure species and hybrids show significant variations at Media v/s Cotton except *D. melanogaster*, F_1B and F_1C (table 3).

Table 3 t-values of larval pupation site preference between sites in pure lines and hybrids

	Glass v/s Media	Glass v/s Cotton	Media v/s Cotton
D. melanogaster [b]	20.583***	34.18***	1.452
D. simulans [a]	82.008***	4.583**	145.962***
D. mauritiana [a]	66.509***	3.498**	111.5***
F_1A	1.211	4.959***	8.815***
F_1B [b]	0.901	1.14	1.861
F_1C	1.696	6.224***	2.755
F_1D	-	-	-
F_1E [a]	71.161**	2.689	111.47***
F_1F	-	-	-

df = 9, *** $P < 0.001$ level, ** $P < 0.01$ level, * $P < 0.05$ level.
[a] - Not significant at glass between them, [b] - Not significant at media between them

DISCUSSION

Larval period is an important stage in life cycle of *Drosophila*, which moves to different surfaces for pupation. One vital aspect of the *Drosophila* life cycle is the ability of the third instar larvae to pupate on substratum since puparia remains immobile and exposed to potentially harmful environmental factors (Manning and Markow, 1981).

The studies on *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. yakuba* showed significant variation in their PSP (Sameoto and Miller, 1968; Shivanna *et al.,* 1996; vandal *et al.,* 2003). Though they are sibling species, *D. melanogaster* contrasts the result of other species. The type of sites selected by larvae affects pupal survival and the choice of pupation sites by larvae have genetic basis. The genetic difference between larvae that prefer to pupate on food and larvae that prefer bottom of the cages is simple and due mainly to a single major gene difference (de Souza *et al.,* 1970).

Manning and Markow, (1981) studied the light dependent PSP in *D. melanogaster*, *D. simulans* and their hybrids. *D. melanogaster* prefers to pupate in dark, while its sibling species *D. simulans* prefers to pupate in light, when the species are tested in isolation, as well as when cultured and tested together. Progeny from the cross of *D. melanogaster* females and *D. simulans* male selected pupation sites exactly intermediate between parents, while the reciprocal cross offsprings preferred light for their pupation.

Present study showed that, *D. melanogaster* preferred to pupate on glass while its sibling species *D. simulans* and *D. mauritiana* preferred to pupate on media. The statistical analysis (One-way ANOVA) showed that there is a significant difference between species at two sites.

The progeny from cross of *D. melanogaster* females and *D. simulans* males showed media pupation more and percentage of PSP is intermediate than the parents. Reciprocal cross progenies are less in number and showed media pupation; it is similar to *D. simulans*. The PSP of hybrid between *D. melanogaster* females and *D. mauritiana* males showed *D. melanogaster* character, which prefers glass for pupation. The progeny of reciprocal cross were not produced. The larvae of *D. simulans* female and *D. mauritiana* male show the maximum media pupation. The percentage of their PSP is more than the parents as they are media pupating species.
Sokolowski and Bauer, (1989) investigated the inheritance of

D. melanogaster larval pupation behaviour in sixteen reciprocal crosses between field collected lines. The chromosomal analysis showed that, the second and third chromosomes act additively on pupation distance and that the third pair of chromosomes had a much larger effect than the second.

Although pupation height in different species of *Drosophila* is affected by several biotic and abiotic factors, a number of studies have been reported the influence of genetic factors on pupation site preference in various *Drosophila* species (de Souza *et al.,* 1970; Bauer and Sokolowski, 1985; 1988; Casares and Carracedo, 1986a and b; Garcia-Florez *et al.,* 1989; Sokolowski and Bauer,1989). Based on the selection experiments, it has been clearly demonstrated that there is substantial amount of additive genetic variation for pupation height in *D. melanogaster*, *D. simulans* and *D. ananassae* (Markow, 1979; Casares and Carracedo, 1986a; Garcia-Florez *et al.,* 1989; Singh and Pandey, 1993b).

The difference in the PSP of *D. melanogaster* is significant with *D. simulans*, *D. mauritiana*, F_1A, F_1B and F_1C at both glass and media except F_1B at media. *D. simulans* and *D. mauritiana* shows significant difference with all except with each other and F_1E at glass. No cotton pupation is found in either of pure species and hybrids. Statistical analysis (Independent t-test) reveals that there is a significant difference between species as well as species – hybrids at two pupation sites.

Bauer and Sokolowski, (1985; 1989) demonstrated the genetic control of larval pupation behaviour by making cross between high and low pupating strains of *D. melanogaster*. A transient maternal effect on pupation behaviour was also detected which was confined to only F_1 backcross.

Paired t-test value (table-3) shows that there is significant difference between different sites of species and hybrids. All 3 species found to be significant at all sites except *D. melanogaster* at Media v/s Cotton and in hybrids, all are significant except F_1A and F_1C at Glass v/s Media, F_1E at Glass v/s Cotton. The larvae of F_1B found to be non-significant with all the three pupation site combinations.

Despite of intermediate nature of pupation; some hybrids (*D. melanogaster* and *D. simulans*) are influenced by the genes of one parent (*D. simulans*) irrespective of male or female. Whereas F_1C hybrids (*D. melanogaster* and *D. mauritiana*), are influenced by the genes of female (*D. melanogaster*) parent.

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