Larval pupation site preference in a few species of *Drosophila*

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Larval pupation site preference (PSP) is an important event in preadult development. The PSP was studied on the basis of the number of larvae pupated at different sites in the cultures. The results revealed that the larval PSP in different species of *Drosophila* varies significantly at different sites. The sympatric species also differ in their PSP and the pupation behaviour does not correspond with the taxonomic relationship between the species.

Keywords: *Drosophila*, Larval pupation, Pupation site preference, Taxonomic relationship

The study of behaviour is relatively new field of investigation and the behaviour genetics largely began as a byproduct of their research investigations in a number of organisms including *Drosophila*. *Drosophila* exhibits four stages in the life cycle, egg, larva, pupae and adult. One vital aspect of the *Drosophila* life cycle is the ability of third instar larvae to pupate on substratum since puparia remains immobile and exposed to potentially harmful environmental factors. The larval pupation site preference (PSP) is an important event in *Drosophila* preadult development, because the place selected by the larva can have decisive influence on their subsequent survival as pupae.

Investigations on pupation site preference in various *Drosophila* species have been made. In most of these studies influence of various factors on PSP has been investigated by measuring the pupation height (the distance a larva pupates above the surface of the food medium). The PSP has also been studied in different species by analysing the percentage of pupae pupated at different sites viz.; cotton, glass and medium in the culture.

The studies among the closely related sibling species belonging to the melanogaster subgroup of *Drosophila*, namely *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. yakuba* showed variations in their PSP. The pupation height has been studied in closely related sympatric species namely *D. ananassae*, *D. bipectinata* and *D. malerkotliana*, showing significant variation in their pupation height. The high and low pupation height selection in *D. ananassae* is under polygenic control with a substantial amount of additive genetic variation. The preliminary studies on larval PSP in *D. ananassae* showed maximum pupation on glass, whereas the closely related sympatric species *D. bipectinata* and *D. malerkotliana* have not been studied.

In order to analyse the relationship, if any, at PSP between the closely related sympatric species such as *D. ananassae*, *D. bipectinata*, *D. malerkotliana* along with other species namely *D. virilis*, *D. novamexicana* and *D. texana* larval PSP in different species of *Drosophila* have been studied.

Following *Drosophila* species were used: *D. ananassae*, *D. bipectinata* and *D. malerkotliana* are closely related sympatric species belongs to ananassae subgroup of the melanogaster species group. *D. virilis*, *D. novamexicana* and *D. texana* are belong to virilis group. In order to maintain uniformity with regard to the density and age of the larvae, the eggs were collected every 6 hours, using the modified technique of Delecour as described by Ramachandra and Ranganath and allowed to hatch. First instar larvae (50) from these cultures were isolated and transferred to vials (10×3.8cm) containing equal quantities of wheat cream agar medium. Extra yeast (50μl/vial) was added everyday to feed the larvae and to maintain the moisture of the culture medium. These cultures (includes 10 replicates) were raised at constant temperature of (22±1°C) with 80% RH. The mean values as well as the percentage of individuals pupated at different sites were calculated on the basis of the number of larvae pupated.
pupated at different sites. The data were subjected to statistical (Student's t-test) analysis to find out the significance of difference between the sites viz: cotton versus glass, glass versus medium and medium versus cotton of all the species. To assess the level of significance of the PSP at three different sites of a species and between species of Drosophila, the data were analyzed by ANOVA.

Drosophila larvae exhibit behaviour such as burrowing, skipping, feeding and pupation site preference. At the end of larval phase, mature third instar larvae form puparium which incorporate the larval skin to the solid surface and the site chosen for pupation by larva is a component of behaviour. In laboratory cultures the PSP of Drosophila varies from species to species, the larvae are seen to pupate in various proportions in the food medium, on glass wall of the culture bottles and in cotton plugs. These pupation sites differ from species to species with respect to surface structure and moisture content.

Table 1 shows the mean and percentage of larvae pupating at all the sites in different species along with the percentage of mortality. Among species analyzed the sympatric species, D. ananassae, D. bipectinata and D. malerkotliana pupates at all the three sites. D. virilis group species D. virilis, D. novamexicana and D. texana pupates at glass and media only. The cotton pupation of D. ananassae is in contrast to the earlier report of shivanna et al 1996. Wherein they reported that D. ananassae pupates only on glass and media. D. bipectinata and D. malerkotliana showed similarity in their pupation behaviour. The comparison between sympatric species showed significant variation in PSP. The studies with respect to pupation height of these 3 species revealed significant variation.

The present analysis showed that the larvae of D. virilis pupate maximum on the glass and minimum in the medium. It is in confirmation with the earlier report that the larvae of D. virilis pupariates on the wall of its container. The analysis of PSP of D. novamexicana and D. texana showed similarity with D. virilis in pupation behaviour, that is all these 3 species pupates maximum on glass and minimum in media and they do not prefer to pupate on cotton. The D. virilis group species exhibits a similar type of pupation behaviour. The nasuta subgroup species namely, D. nasuta nasuta, D. n. albomicans, D. n. kepalauana, D. sulfurigaster sulfurigaster D.s.neonasuta, are similar in their percentage of pupation at glass and media, and revealed nonsignificant variation with respect to their pupation behaviour. Student's t test (Table 1) reveals that the PSP between glass and media of all species is highly significant, between glass and cotton of all species showed highly significant variation except D. bipectinata and D. malerkotliana whereas these two species showed significant variation between media and cotton pupation compared to all other species. The statistical analysis (ANOVA) revealed that the PSP variation between sites of all these species is highly significant (Table 2), between species and sites also it is found to be significant (df = 2, df = 27 F = 5.0365 P < 0.05%).

The studies among the sibling species, D. melanogaster, D. simulans, D. yakuba and D. mauritiana revealed that the sibling species showed significant variation in PSP. The studies with respect to pupation height of these species revealed significant variation.

### Table 1: Pupation site preference in different species of Drosophila larvae

<table>
<thead>
<tr>
<th>Species</th>
<th>Cotton</th>
<th>C×G**</th>
<th>Glass</th>
<th>G×M**</th>
<th>Media</th>
<th>M×C**</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. ananassae</td>
<td>5.1 ± 5.17</td>
<td>7.580*</td>
<td>39 ± 5.03</td>
<td>7.692*</td>
<td>4.6 ± 2.59</td>
<td>0.118</td>
<td>2.6</td>
</tr>
<tr>
<td>D. bipectinata</td>
<td>1.8 ± 3.01</td>
<td>0.022</td>
<td>1.9 ± 1.66</td>
<td>9.595*</td>
<td>44.8 ± 3.93</td>
<td>9.516*</td>
<td>3</td>
</tr>
<tr>
<td>D. malerkotliana</td>
<td>1.8 ± 4.39</td>
<td>1.475</td>
<td>8.4 ± 4.71</td>
<td>6.395*</td>
<td>37 ± 5.57</td>
<td>7.871*</td>
<td>5.2</td>
</tr>
<tr>
<td>D. virilis</td>
<td>0.0 ± 0.00</td>
<td>10.621*</td>
<td>47.5 ± 1.26</td>
<td>10.263*</td>
<td>1.6 ± 0.96</td>
<td>0.357</td>
<td>1.8</td>
</tr>
<tr>
<td>D. novamexicana</td>
<td>0.0 ± 0.00</td>
<td>8.564*</td>
<td>38.3 ± 4.52</td>
<td>6.507*</td>
<td>9.2 ± 4.13</td>
<td>2.057</td>
<td>5</td>
</tr>
<tr>
<td>D. texana</td>
<td>0.0 ± 0.00</td>
<td>8.676*</td>
<td>38.8 ± 1.22</td>
<td>6.641*</td>
<td>9.1 ± 2.23</td>
<td>2.034</td>
<td>4.2</td>
</tr>
</tbody>
</table>

N = 10 df = 18 P < 0.001 *Significant **Student's t-test C-Cotton M-Media G-Glass
and the present study on sympatric species D. anamassae, D. bipectinata and D. malerkolliana showed significant variation in their pupation behaviour, whereas the D. nasuta subgroup species and D. virilis group species, D. virilis, D. novamexicana and D. texana showed nonsignificant variations in their PSP even though they are not sibling or sympatric species.

The present study revealed that the pupation behaviour of D. bipectinata, D. malerkolliana are similar in their PSP at different sites and D. anamassae, D. virilis, D. texana and D. novamexicana are also showed similarity in their percentage of pupation. These results confirms the earlier studies. The pattern of pupation of D. bipectinata and D. malerkolliana and D. anamassae, D. virilis, D. texana and D. novamexicana confirms and resembles the pattern I & II of phylogenetic relationship pattern proposed/reported by Shivanna et al. The statistical analysis revealed significant variation at different sites and between species eventhough they are closely related sympatric species and the PSP pattern does not coincides with the taxonomic relationship.

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References
5 Markow T A, Survey of intra and inter specific variation for pupation height in Drosophila, Behav Genet, 9 (1979) 209.
10 Barker J S F, Ecological differences and competitive interaction between D.melanogaster and D.simulans in small laboratory populations, Oecologia, 8 (1971) 139.
13 Shivanna N & Ramesh S R, Intraspecific larval pupation site preference in D.melanogaster, Dros Inf Serv, 80 (1997) 2.
14 Stalker H D, Sexual isolation studies in the species complex Drosophila virilis Genetics, 42 (1942) 238.