Effect of larval size and weight on pupation site preference in different species of *Drosophila*.

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The larval pupation site preference (PSP) is an important event in *Drosophila* preadult development, because the place selected by the larva has decisive influence on the subsequent survival as pupae (Sameoto and Miller, 1968). The larval PSP has been analyzed by two types of phenotypic characters, the pupation height and pupation site preference. The pupation height has been studied by measuring the distance a larva pupated above the surface of the food medium (Sokal *et al.,* 1960; Sokolowski, 1985; Casares and Carracedo, 1987; Schnebel and Grossfield, 1992; Singh and Pandey, 1993). The larval PSP has also been analyzed by measuring the percentage of larva pupated at different sites *viz*; cotton, glass and medium and revealed that most of the *Drosophila* species prefer maximum media and few species prefer glass and cotton for pupation (Barker, 1971; Shirk *et al.,* 1988; Shivanna *et al.,* 1996; Shivanna and Ramesh, 1997; Vandal *et al.,* 2003).

The effect of abiotic and biotic factors on pupation height has been studied in different species of *Drosophila* (de Souza *et al.,* 1970; Sokolowski, 1985; Mueller and Sweet, 1986; Godoy-Herrera, 1986; Casares and Carracedo, 1987; Rodriguez and Sokolowski, 1987; Schnebel and Grossfield, 1992; Pandey and Singh, 1993; Joshi and Mueller, 1993; Hodge *et al.,* 1996; Hodge and Caslaw, 1997; Joshi, 1997) and it reveals that these factors influence the pupation height.

The studies of Shivanna and Ramesh (1995) on larval salivary gland secretions (glue proteins) and the gland size in 15 species of *Drosophila* reveal that the quantity of secretions synthesized is independent of size of the salivary glands. The correlation studies between larval PSP and the quantity of larval salivary gland protein called glue protein revealed that the larvae which secrete a larger quantity of glue protein tend to pupate on media and those that synthesize half of the quantity of glue protein prefer to pupate on glass wall, and very low or negligible quantity of glue protein prefer to pupate on cotton (Shivanna *et al.,* 1996). But the importance of size and weight of the larva in relation to PSP has not been studied. The present study was undertaken to analyze whether there is any relation between the size and weight of larvae and their pupation site preference.

The following *Drosophila* species were used to study the relationship between size and weight of the larva on PSP. *D. melanogaster*, *D. simulans, D. yakuba* and *D. mauritiana* are sibling species belonging to the *melanogaster* subgroup species. *D. ananassae, D. bipectinata, D. malerkotliana* and *D. rajasekari* are closely related sympatric species and belong to *ananassae* subgroup of *melanogaster* species group. *D. virilis* and *D. novamexicana* belong to the *virilis* group and *D. hydei* belongs to *repleta* species group (Bock and Wheeler, 1972; Ehrman, 1978; Ranganath *et al.,* 1985; Ashburner, 1989; Singh and Pandey, 1991).

In order to maintain uniformity with regard to the age of the larvae, the eggs were collected every 6 hours using the modified technique of Delcour as described by Ramachandra and Ranganath (1988) and allowed to hatch. The culture was maintained at 22±1°C with 80% RH. Late third instar larvae (96 hour old) were isolated from the cultures, and the size of the larva was measured from the anterior spiracles to the posterior spiracles (Length and Breadth). Using an electronic balance, the weight of the larva was quantified for each individual (twenty five larvae). Then the larvae were

allowed to pupate in the culture vials. The partial correlation analysis was used to correlate the size and weight of the larva with PSP.

Table 1 shows the mean of larval size (length and breadth) weight and percentage of PSP in different species of *Drosophila*. Among the media-pupating species, *D. simulans, D. yakuba, D. mauritiana, D. bipectinata* and *D. malerkotliana* prefer maximum media for pupation (93.4%, 71.4%, 53.2%, 86.6% and 74%, respectively). The approximate larval size of these species varies between 3 to 3.5 mm and weight varies between 0.6 to 0.7 mg. The larvae of *D. melanogaster, D. ananassae, D. virilis, D. novamexicana,* and *D. hydei* prefer maximum glass for pupation (94.2%, 78%, 95%, 76.6% and 74.25, respectively). The larval size of these species varies between 3.3 to 6 mm and weight varies between 0.6 to 1.7 mg. The larvae of *D. rajasekari* prefer maximum cotton for pupation (61.8%). The larval size is 3.5 mm and weight is 0.6 mg.

Table 1. Size and weight of third instar larvae and their maximum PSP in different species of *Drosophila*.

Species	Size (LXB) (mm)	Weight (mg)	Percentage of pupation (%)
Media pupating species			
D.simulans	3.3	0.6	93.4
D.mauritiana	3.5	0.6	71.4
D.yakuba	3.4	0.6	53.2
D.bipectinata	3.5	0.7	86.6
D.malerkotliana	3.0	0.7	74.0
Glass pupating species			
D. melanogaster	3.3	0.6	94.2
D.ananassae	3.5	0.7	78.0
D. virilis	5.0	1.1	95.0
D. novamexicana	6.0	1.7	76.6
D.hydei	5.5	1.7	74.2
Cotton pupating species			
D.rajasekari	3.5	0.6	61.8

Size- Mean length and breadth of larvae includes anterior and posterior spiracles (mm). Weight- Mean weight of late third instars larvae (mg).

The comparison between the larval size and weight with maximum PSP in different species analyzed shows significantly negative correlation (partial correlation) with their maximum PSP; on media *D. simulans* (r = -0.9545 and r = -0.9981), *D. yakuba* (r = -0.9559 and r = -0.9965), *D. mauritiana* (r = -0.9065 and r = -0.9973), *D. bipectinata* (r = -0.9644 and r = -0.9971) and *D. malerkotliana* (r = -0.9979 and r = -0.9458), on glass *D. melanogaster* (r = -0.9605 and r = -0.9984), *D. ananassae* (r = -0.9661 and r = -0.9961), *D. virilis* (r = -0.983 and r = -0.9439), *D. novamexicana* (r = -0.97 and r = -0.9945) and *D. hydei* (r = -0.9769 and r = -0.9924) and on cotton *D. rajasekari* (r = -0.9165 and r = -0.9977).

The larvae of different species show differences in the larval size and weight with their pupation site preference. The glass pupating species *D. virilis*, *D. novamexicana* and *D. hydei* are larger in size and weight than media pupating species, *D. simulans, D. yakuba, D. mauritiana, D. bipectinata,* and *D. malerkotliana*. The larvae of glass pupating species *D. melanogaster* and *D. ananassae* and cotton pupating species *D. rajasekari* are more or less similar in their size and weight. In contrast, *D. virilis, D. novamexicana* and *D. hydei* have bigger size than the larvae of *D. melanogaster*, *D. ananassae,* and *D. rajasekari,* but they prefer to pupate on the glass. The culture conditions were maintained constant for all the species analyzed.

The larval developmental period is determined by the time needed to reach the critical weight, and the time from the critical stage to pupation and final body weight is determined by the critical body weight and the possibilities for additional growth before the onset of pupariation as determined by the availability of resources (Robertson, 1963). The hormonal event leading pupariation are initiated in the third larval instar when critical stage is reached, after which there is a fixed period of post critical feeding growth before pupariation occurs (Riddiford, 1985). In *D. melanogaster*, the critical stage occurs right after the second moult. The size of the larva reaching the critical stage of

commitment to pupariation is referred to as its critical weight. The critical weight is a symptom of the underlying physiology and need not imply a direct relation between size and decision to pupate. Larval critical weight will partly determine the way age and size at maturity respond to environmental variations and is, therefore, important life history evolution (Bernardo, 1993). The studies of larval size, developmental stage, and age on pupation at different temperatures in different populations of *D. melanogaster* reveal that the weight of the larva within the age is significantly correlated with pupation probability in tropical populations, whereas in temperate populations no relation with larval weight, age, and with pupation probability, and most of the larvae succeeded in pupating on/in and then produced small adults (Bochdanovitz and de Jong, 2003). The same larval weight or age might have different meaning for different genotype, and higher probability of pupating was associated with lower adult size once feeding was stopped. Minimal size is needed to pupate, and that might vary between genotypes within populations of *Drosophila* (Bakker, 1961; Bochdanovitz and de Jong, 2003).

Shivanna and Ramesh (1995) studied the larval salivary gland and quantity of glue protein and reported that the secretion of larval glue protein is not associated with size of the salivary gland, and quantity of the secretion is independent of the salivary gland. The larvae which secrete a larger quantity of glue protein tend to pupate on media, and lesser preferred glass for pupation. It reveals that the larval PSP depends on the quantity of glue protein synthesized by the late third instar larvae (Shivanna *et al.,* 1996). The present study reveals that, irrespective of size and weight of the larvae, the species *D. melanogaster, D. ananassae, D. virilis, D. novamexicana* and *D. hydei* prefer to pupate on glass. *D. rajasekari* having similar size and weight with glass and media pupating species prefer to pupate on cotton. *D. virilis, D. novamexicana* and *D. hydei* have more size and weight than other species and prefer to pupate on glass. The *melanogaster* and *ananassae* group species larvae were found to pupate on glass and media though they have similar size and weight. The result shows that the pupation site preference is not taxonomically related. Further it is concluded on the basis of the above result that the size and weight of the larvae has no relationship with their pupation site choice.

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Revised list of drosophilid species so far described and recorded from the Kumaon region, Uttarakhand State, India, with replacement names for two homonyms.

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Introduction

The Cytogenetics Laboratory in the Department of Zoology, Kumaon University, Nainital, India, is actively exploring the Drosophilid fauna of the Kumaon region since 1984, which was completely unknown for its drosophilid fauna. Since then a number of papers have been published on Kumaon Drosophilidae (Singh and Negi, 1989, 1992, 1995; Singh and Dash, 1993, 1998; Singh, Dash and Fartyal, 2000, 2004; Singh and Fartyal, 2002; Fartyal, Singh and Toda, 2005; Joshi, Fartyal and Singh, 2005; Upadhyay and Singh, 2006). The area is characterized by having dense evergreen coniferous forests with medium to very steep slopes and extremely moist condition due to heavy rain fall. This paper deals with the list of drosophilid species so far described and recorded from the Kumaon region, India, with replacement names for two homonyms, *i.e*. *Drosophila* (*Drosophila*) *kulouriensis*; old name e*longata* Singh, Dash and Fartyal, 2004, (Ref. Senck. Biol., 83(2): 173, 2004), and *Drosophila (Drosophila*) *sattalensis*; old name *serrata* Singh, Dash and Fartyal, 2004, (Ref. Senck. Biol., 83(2): 176, 2004).

Materials and Methods

This list is the result of drosophilid collections undertaken by the students of the Cytogenetics Laboratory, Department of Zoology, Kumaon University, Nainital, India, since 1984. The collections were largely made by net sweeping over wild vegetation and by trap baiting. The collected flies were preserved in 70% alcohol and stored for their head, thorax and abdomen.

Observations

Out of 82 species collected, a total of 37 species belonging to different genera of the family Drosophilidae were described as new to science, and 18 species were recorded for the first time from India since 1984 from the Kumaon region, India. The genera *Lordiphosa*, Malloch and