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EFFECT OF TEMPERATURE AND DENSITY ON LARVAL PUPATION SITE PREFERENCE IN DIFFERENT SPECIES OF *DROSOPHILA*

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Effect of temperature and density on larval pupation site preference in different species of *Drosophila*. – **Vandal N.B., Siddalingamurthy G.S., and Shivanna N. –** The larval pupation site preferences (PSP) in different species of *Drosophila* were studied at varied larval density and temperatures. Compared to control, at highest larval densities, the percentage of media pupation decreased and glass pupation increased at lowest temperatures in *D. yakuba,* whereas at highest temperatures with lowest larval density the media pupation increased. At all the larval densities studied; in *D. mauritiana* the glass and media pupation increased at 20°C, whereas at highest temperatures the media pupation increased and the glass pupation decreased, in *D. rajasekari* the cotton pupation and in *D. virilis* the glass pupation decreased and the media pupation increased at lowest and highest temperatures, the larvae of *D. novamexicana* prefer to pupate maximum on glass and minimum on media at lowest temperatures and at highest temperatures the pupation increased on glass and decreased on the media. In *D. hydei* the glass and media pupation decreased at lowest temperatures, and increased glass and decreased the media pupation at highest temperatures with highest and lowest density respectively. Statistical analysis has revealed that the variation of PSP in all the species with temperature, density, and sites is significant. These results show that at lowest and highest temperature and larval density the PSP significantly differ in all the species of *Drosophila* analyzed.

Key words: Drosophila, pupation site preference, density, temperature, larva, habitat choice.

Влияние температуры и плотности на выбор мест окукливания личинками дрозофил. – Вандал Н.Б., Сиддалингамурти Дж.С., Шиванна Н. – Изучен выбор мест окукливания (ВМО) личинками дрозофил при различных показателях плотности личинок и температуре. По сравнению с контрольной группой, при наивысшей плотности личинок процент окукливания на среде уменьшался, а на стекле усиливался при низких температурах для *D. yakuba*. Напротив, при высоких температурах с низкой плотностью личинок окукливание на среде усиливалось. При всех изученных плотностях личинок наблюдалось следующее: для *D. mauritiana* окукливание на стекле и на среде усиливалось при 20°C, в то время как при высоких температурах окукливание на среде увеличивалось с ослаблением окукливания на стекле; для *D. rajasekari* окукливание на хлопке и для *D. virilis* окукливание на стекле уменьшались, а окукливание на среде возрастало при низких и высоких температурах соответственно; личинки *D. novamexicana* окукливались больше на стекле и меньше на среде при низких температурах, а при высоких температурах их окукливание усиливалось на стекле и уменьшалось на среде. Для *D. hydei* окукливание на стекле и на среде уменьшалось при низких температурах, а при высоких температурах оно увеличивалось на стекле и уменьшалось на среде при самой высокой и самой низкой плотности соответственно. Статистический анализ показал, что изменения ВМО по температуре, плотности и месту окукливания были значимыми у всех видов Drosophila. Таким образом, при низкой и высокой температурах и плотностях личинок ВМО достоверно отличается у всех исследованных видов дрозофил.

Ключевые слова: Drosophila, предпочтение мест окукливания, плотность, температура, личинка, биотопические предпочтения.

Temperature and density both have major phenotypic effects on the life-history traits in *Drosophila melanogaster* (Prasad, Joshi, 2003). The effect of temperature has been studied in different species of *Drosophila* on both adult and preadult characters. Compared to individuals reared at the standard temperature of about 25°C, rearing at a colder temperature (16 to 17°C) results in an increased egg size as well as an increased larval and pupal duration, mature larval size and adult size at eclosion, larger wing size, larval critical weight, life span, life time fecundity and progeny production but this reduces daily fecundity (David et al., 1994; Delpuech et al., 1995; Partridge et al., 1995; Noach et al., 1996; Crill et al., 1996; Lazebry et al., 1996; de Moed et al., 1997, 1999; Imasheva et al., 1997; French et al., 1998; Eanes, 1999; Blankenhorn, 1999; Robinson, Partridge, 2001; Bochdanovits et al., 2003).

Increased larval crowding in laboratory cultures results in a decrease in the food available over time and an increase in the metabolic waste levels, especially that of ammonia (Borash et al., 1998). The major phenotypic effects of rearing larvae at high (several hundreds per vial) versus moderate (50 to 100 per vial) density are an increased larval and pupal mortality, larval development time, pupation height and adult life span as well as reduced adult size and fecundity (Mueller, 1986, 1997; Guo et al., 1991; Zwaan et al., 1991; Mueller et al., 1993; Roper et al., 1996; Chippindale et al., 1997; Joshi, Mueller, 1997; Santos et al., 1997; Shiotsugu et al., 1997; Sokolowski et al., 1997; Mueller, Joshi, 2000; Prasad et al., 2001).

Drosophila species usually show less specificity in their adaptations for larval sites than for oviposition sites, and the adaptive differentiation in response to temperature stress among a population of *D. pseudoobscura* is more pronounced for puparia than for adults (Kaneshiro et al., 1973; Coyene et al., 1983). Joshi (1997) reviewed the obvious effects of density on the adaptations of both larva and adult. The food medium rapidly becomes very moist and soggy in crowded cultures. In such cultures, individuals pupating on or close to the surface of the medium have an increased chance of being dislodged and drowned in the medium. 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 its subsequent survival as a pupa (Sameoto, Miller, 1968). De Souza et al. (1968) has reported that the simple genetic control for pupation site choice in *D. willistoni* under high-density conditions clearly implicates a different type of behaviour than the pupation height measured for *D. melanogaster* and *D. simulans* (Sokal et al., 1960; Sameoto, Miller, 1968).

The PSP has been analyzed by two types of phenotypic characters, one is the pupation height and the other is pupation site preference. The pupation height studies have been made by using different factors such as moisture, light, temperature, density, sex, larval developmental time, selection for high and low pupation height and its genetic control (Sokal et al., 1960; Mensua, 1967; Sameoto, Miller, 1968; Markow, 1979; Fogleman, Markow, 1982; Ringo, Wood, 1983; Bauer, Sokolowski, 1985; Casares, Carracedo, 1987; Singh, Pandey, 1993 *a*, *b*; Pandey, Singh, 1993). An environmental variable known to influence pupation in *Drosophila* is temperature. Species differences in the ability to pupate successfully, as well as in pupation height choice have been shown to be temperature dependent (Grossfield, 1978; J.A. McKenzie, S.W. McKenzie, 1979;

Fogleman, Markow, 1982; Schnebel, Grossfield, 1986; Kimura, 1988; Schnebel, Grossfield, 1992).

The PSP has also been studied by analyzing the percentage of pupae pupated at different sites viz; cotton, glass and the medium in the culture. The studies of Barker (1971), Shirk et al. (1988), Shivanna et al. (1996), Vandal et al. (2003), Vandal and Shivanna (2004, 2005 *a*, *b*, 2006, 2007) have reported that most of the species [*D. simulans*, *D. gibberosa*, *D. bipectinata*, *D. malerkotliana*, *D. yakuba*, *D. mauritiana*, *nasuta* subgroup species (*D. nasuta nasuta*, *D. nasuta albomicans*, *D. nasuta kepulauana*, *D. sulfurigaster sulfurigaster*, *D. sulfurigaster neonasuta*), *D. immigrans*, *D. rubida* and *D. pararubida*] under normal conditions prefer to pupate maximum on the media. The quantity of larval salivary gland protein, larval locomotory path length, pattern, temperature and substrates plays a role in larval pupation site preference in *Drosophila*. Larval PSP has not been studied using combined factors like temperature and density. In view of this the present study was undertaken to know the effect of temperature along with density on larval PSP in *Drosophila* species occupying different sites for pupation at constant conditions.

MATERIALS AND METHODS

For the present investigation, closely related sibling species were taken to study the effect of temperature with varying larval densities on PSP: *D. yakuba* and *D. mauritiana* belong to *melanogaster* subgroup species, *D. rajasekari* is a closely related sympatric species belonging to *suzukii* subgroup, *D. virilis* and *D. novamexicana* belong to *virilis* group and *D. hydei* belongs to *repleta* group (Bock, Wheeler, 1972; Ehrman, 1978; Ranganath et al., 1985; Ashburner, 1989). These *Drosophila* species were collected from the *Drosophila* stock centre, University of Mysore, Mysore, India, maintained about 20 years.

In order to maintain uniformity with regard to age of the larvae the eggs were collected every 6 hours using a modified technique of Delcour described by Ramachandra and Ranganath, (1988) and allowed to hatch. First instar larvae – about 50 (control 22±1°C), 100, 150, 200, and 250 from the cultures were isolated and transferred to a vial (10 × 3.8 cm) containing equal quantities of the wheat cream agar medium (Shivanna et al., 1996). About 50 μl of dilute yeast was added everyday to feed the larvae and to maintain the moisture content of the food medium. The cultures were kept at four different temperatures viz; 15, 20, 25, and 30°C.

Ten replicates were carried out for each experiment. The mean values as well as the percentage of pupation were calculated based on the number of larvae pupated at different sites viz; cotton, glass, and the medium. Primary data (the number of pupae on different sites) were subjected to three-way ANOVA (temperature × density × sites) to analyze the combined effect of temperature and density at three different sites of *Drosophila*.

RESULTS

Figure 1 (1*a*, 2*a* and 3*a*) reveals that the percentage of cotton pupation is minimum at all the temperatures and densities in *D. yakuba* and *D. mauritiana*. Compared to control, the cotton pupation decreased at all the larval densities and at different temperatures in *D. mauritiana* whereas it increased at 15ºC with 100 to 250 larvae/vial and 25ºC with

100 and 250 larvae/vial. *D. rajasekari* prefers to pupate maximum on cotton at all the temperatures at varied densities. Compared to control, the larval pupation on cotton decreased at all the larval densities and at different temperatures except at 20ºC, wherein the cotton pupation increased with 100 to 250 larvae/vial.

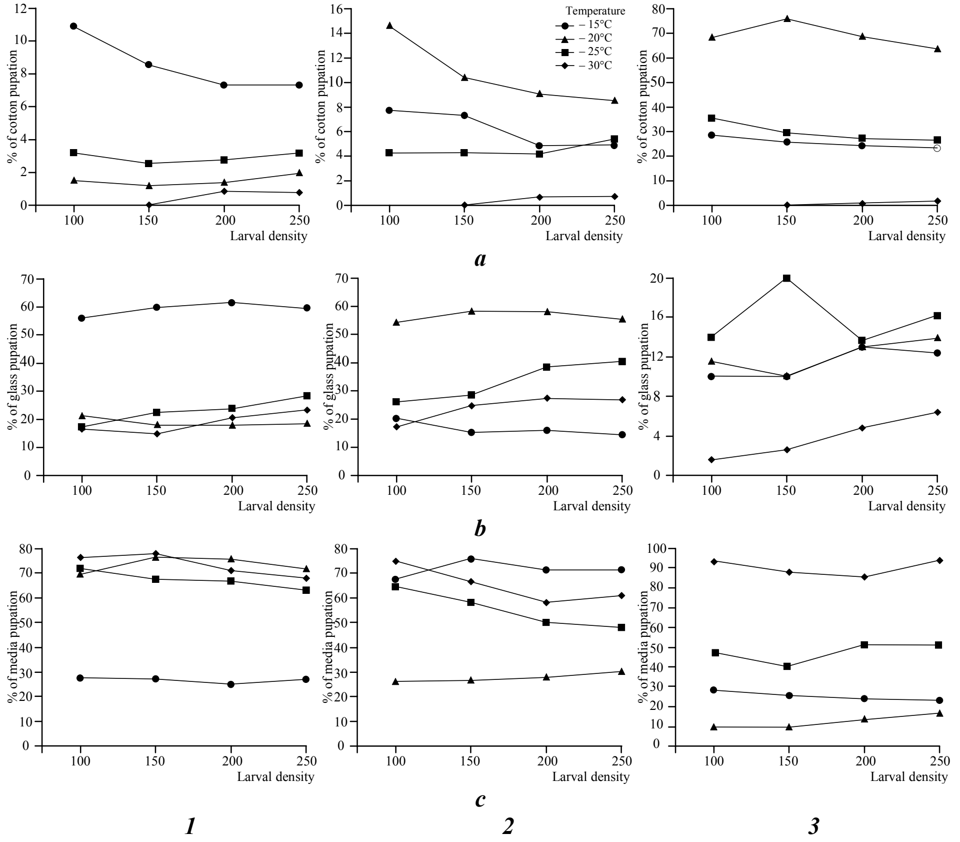

Fig. 1. Percentage of larval pupation site preference at different temperatures and densities in *Drosophila*: *1 – D. yakuba*, *2 – D. mauritiana*, *3 – D. rajasekari*; *a* – cotton, *b* – glass, *c* – media

Figure 1 (1*b*, 2*b*, and 3*b*) shows the mean percentage of glass pupation in *D. yakuba*, *D. mauritiana* and *D. rajasekari* at different larval densities and temperatures. The larvae of *D. yakuba* and *D. mauritiana* prefer to pupate minimum on glass at all the temperatures and larval densities. The glass pupation at 15ºC increases in *D. yakuba*, whereas in *D. mauritiana* it increases at 20ºC with 100 to 250 larvae/vial compared to control. The larvae of sympatric species of *D. rajasekari* prefer to pupate more on glass at 15ºC with 200 and 250 larvae/vial, 20ºC with 100, 200, and 250 larvae/vial and 25ºC with 100 to 250 larvae/vial than control.

Figure 1 (1*c*, 2*c*, and 3*c*) shows the mean percentage of media pupation in *D. yakuba*, *D. mauritiana*, and *D. rajasekari* at different larval densities and temperatures. It reveals that the larvae of *D. yakuba* and *D. mauritiana* prefer to pupate maximum on the media at varying densities and temperatures. Compared to control, *D. yakuba* larvae prefer the media for pupation at 25ºC with 150 and 200 larvae/vial and at 30ºC with 100 and 150 larvae/vial. Whereas *D. mauritiana* prefers it at 20, 30ºC with 100 to 250 larvae / vial and at 25ºC with 100 and 150 larvae/vial. The larvae of *D. rajasekari* prefer to pupate minimum on media at varying densities and temperatures. Compared to control, the media pupation increased at 15, 25, and 30ºC with an increase from 100 to 250 larvae / vial.

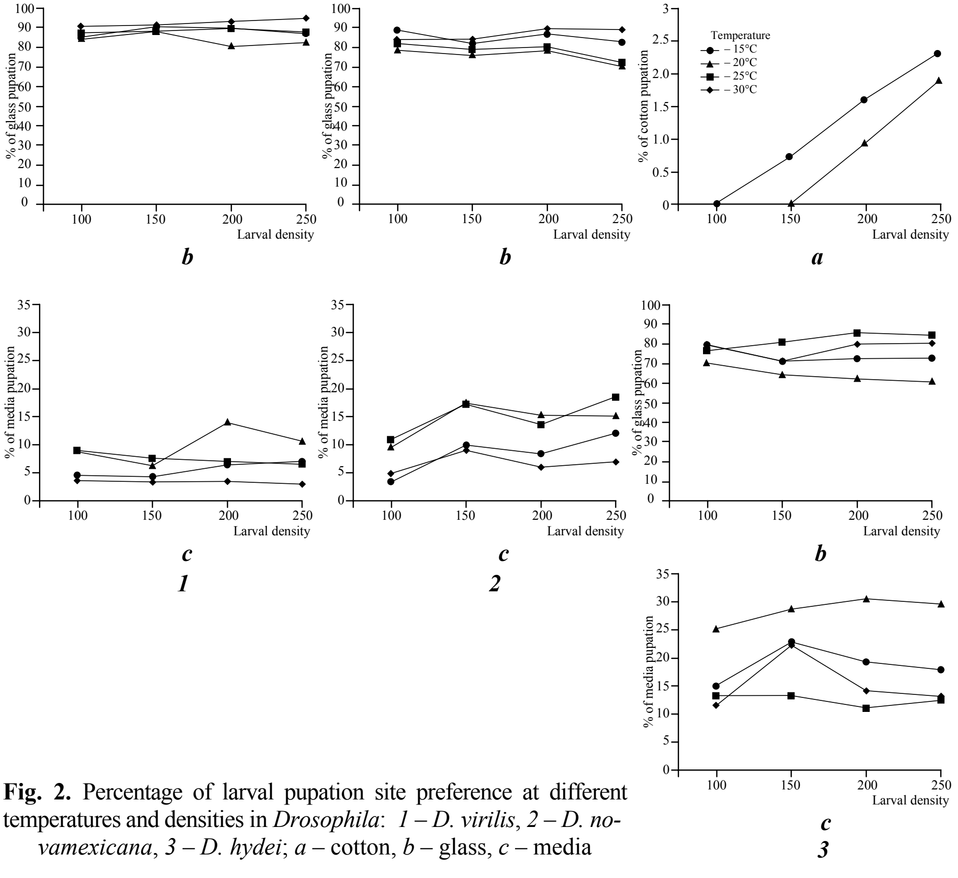

Fig. 2. Percentage of larval pupation site preference at different temperatures and densities in *Drosophila*: *1 – D. virilis*, *2 – D. novamexicana*, *3 – D. hydei*; *a* – cotton, *b* – glass, *c* – media

Figure 2 (3*a*) shows the mean percentage of cotton pupation in *D. hydei*. Compared to control, the larvae prefer cotton for pupation (0.73%, 1.6% and 2.32%) at 15ºC with 150 to 250 larvae/vial and (0.95% and 1.88%) at 30ºC with 200 and 250 larvae/vial. In control, *D. virilis*, *D. novamexicana* and *D. hydei* do not prefer to pupate on cotton.

Figure 2 (1*b*, 2*b*, and 3*b*) shows the mean percentage of glass pupation in *D. virilis*, *D. novamexicana*, and *D. hydei* at different larval densities and temperatures. It reveals that *D. virilis*, *D. novamexicana*, and *D. hydei* larvae prefer to pupate maximum on the media at varying densities and different temperatures. Compared to control, the larvae of *D. virilis* decrease the glass pupation at all the temperatures with a varied larval density. Whereas the larvae of *D. novamexicana* increase their glass pupation at 15 and 30ºC with 100 to 250 larvae/vial, at 25ºC with 100 to 200 larvae/vial and 20ºC with 100 and 200 larvae/vial. The larvae of *D. hydei* increase the glass pupation at 25ºC with 100 to 250 larvae/vial, 30ºC with 100, 200, and 250 larvae/vial and at 15ºC with 100 larvae/vial.

Figure 2 (1*c*, 2*c*, and 3*c*) shows the mean percentage of media pupation in *D. virilis*, *D. novamexicana*, and *D. hydei* at different larval densities and temperatures. Compared to control, the larvae of *D. virilis* increase their media pupation at all the temperatures and a varied larval density except at 30ºC with 250 larvae/vial. The larvae of *D. novamexicana* and *D. hydei* decrease their media pupation at all the temperatures with a varying larval density except *D. novamexicana* at 25ºC with 200 larvae/vial and *D. hydei* at 20ºC with 100 and 250 larvae/vial.

The highest mortality is found to be in *D. yakuba* (7.1, 7.26, 7.75, and 7.8%) at all the densities 100, 150, 200, and 250 larvae/vial at 30ºC and (7.8, and 7.53%) with 100 and 150 larvae/vial at 25ºC. Followed by *D. mauritiana* (9.36%) with 250 larvae/vial at 15ºC, (9 and 7.55%) with 150 and 200 larvae/vial at 25ºC, and (13.6 and 11.4%) with 200 and 250 larvae/vial at 30ºC. *D. rajasekari* (20.8, 25.7 and 33.68%) with 150 to 250 larvae/vial at 15ºC, (10.4%) with 100 larvae/vial at 20ºC and (10.3%) with 150 larvae/vial at 25ºC. *D. virilis* (10.5%) with 100 larvae/vial at 15ºC. *D. novamexicana* (8%) with larvae/vial at 15ºC, (11.6 and 14%) with 100 and 250 larvae/vial at 20ºC, (9.4%) with 250 larvae/vial at 25ºC, and (11%) with 100 larvae/vial at 30ºC. *D. hydei* (7.04 and 7.8%) with 250 larvae/vial at 15 and 20ºC and (8.2% and 8.8%) with 100 larvae/vial at 25 and 30ºC compared to control (table 1).

Table 1

Percentage of larval mortality with varying larval densities at different temperatures in different species of *Drosophila*

Species, larvae/vial	15ºC	20ºC	25ºC	30ºC
1	2	3	4	5
D. yakuba (Control, nil)				
100	5.8	7.3	7.8	7.1
150	4.4	4.4	7.53	7.26
200	6.7	4.8	6.8	7.75
250	6.16	7.64	5.44	7.8
D. mauritiana (Control, nil)				
100	4.9	4.9	5.2	8.0
150	1.6	4.6	9.0	8.66
200	7.5	4.75	7.55	13.6
250	9.36	5.8	6.36	11.4

Table 1 (continuation)

1	2	3	4	5
D. rajasekar (Control, nil)				
100	6.3	10.4	4.5	5.1
150	20.8	4.13	10.33	3.86
200	25.7	4.75	7.75	6.3
250	33.68	5.72	6.32	6.48
D. virilis (Control, 1.8)				
100	10.5	6.9	3.9	5.6
150	5.66	5.0	4.66	5.34
200	3.6	5.35	3.2	3.5
250	5.8	6.8	6.08	2.24
D. novamexicana (Control, 5.0)				
100	5.6	11.6	7.2	11.0
150	8.0	6.2	4.4	6.74
200	4.85	5.8	6.55	4.1
250	5.12	14.0	9.4	3.84
D. hydei (Control, nil)				
100	5.6	4.2	8.2	8.8
150	5.26	6.86	5.8	6.73
200	6.4	6.0	3.6	6.0
250	7.04	7.8	2.8	6.48

Three-way ANOVA on the combined effect of temperature and densities data at three different sites viz; cotton, glass and media pupation has revealed that the variation of larval pupation site preference in all the species and sites are significant except *D. yakuba* on cotton, *D. rajasekari* on the media, *D. virilis* and *D. novamexicana* on glass (table 2).

Table 2

Three-way analysis of variance of pupation site preference
at three different sites in *Drosophila*

Species	Source of variation	Degrees of freedom	*F*- value	*p*-value
D. yakuba	Temperature × Density × Glass	10	78.334*	<0.001
	Temperature × Density × Media	10	76.913*	<0.001
	Temperature × Density × Cotton	10	3.170	<0.001
D. mauritiana	Temperature × Density × Glass	10	66.932*	<0.001
	Temperature × Density × Media	10	48.809*	<0.001
	Temperature × Density × Cotton	10	6.358*	<0.001
D. rajasekari	Temperature × Density × Glass	10	13.030*	<0.001
	Temperature × Density × Media	10	1.286	<0.001
	Temperature × Density × Cotton	10	133.83*	<0.001
D. virilis	Temperature × Density × Glass	10	1.489	<0.001
	Temperature × Density × Media	10	13.083*	<0.001
D. novamexicana	Temperature × Density × Glass	10	2.210	<0.001
	Temperature × Density × Media	10	5.516*	<0.001
D. hydei	Temperature × Density × Glass	10	24.631*	<0.001
	Temperature × Density × Media	10	10.444*	<0.001
	Temperature × Density × Cotton	10	26.068*	<0.001

*Significant.

DISCUSSION

The environmental conditions at which a species can carry out its vital life-history stages will directly influence its geographical and habitat distribution. Conditions that influence the pre-adult stages can be just as important as those affecting adults. Since insect pupae are immobile, they can remain exposed to potentially harmful biotic and abiotic factors for varied periods of time (desiccation, predation, infection etc.). Pupation site choice can be critical for survival. Differences in pupation site choice can reflect the ability of larvae to find food, niche, and competition in general.

In *Drosophila*, temperature is the most important environmental factor which affects all the biological processes at the molecular, cellular, and organismic levels (David et al., 1983). Different strains of *D. nasuta nasuta* showed intraspecific variations with respect to preadult fitness at constant and ambient temperatures (Ashadevi, Ramesh, 1998). Temperature involves limitations on the *Drosophila* activity while temperature sensitivity involves behavioural alterations, which are expressed only at or above a critical temperature. Different species show different optimum temperatures for growing, and some cannot be grown above a certain temperature (within 16 – 30°C), depending on the species, the flies moved to the cooler end of the tube at 22°C when the temperature was above 41ºC (Grossfield, 1978). High temperatures and dry periods for several days may act as a strong selective force on developing pupae (Tonzetich, Ward, 1972). Spassky (1951) found that a particular homokaryotype of *D. pseudoobscura* showed a higher viability on wet food at high temperatures whereas other ones had a higher viability on dry food at lower temperatures. The pupal survivorship decreases at lower temperatures than at higher temperatures in *D. melanica* (Tonzetich, Ward, 1972). Within a stressful temperature range (30 to 34ºC), the adults of the sibling species *D. simulans* flies died after 15 hours at 32ºC, those of *D. melanogaster* being low after 24 hours. At 28ºC, no death occurred for both the species after 15 hours (Parson, 1978).

The larval pupation site preference (PSP) is one of the behaviour of late third instar larvae in *Drosophila.* It has been analyzed by two types of phenotypic characters, one is the pupation height and the other is pupation site preference. The pupation height is the distance the larva moved upward and pupated above the food media. PSP is the percentage of larvae moved / not moved upward and pupated at different sites. The larval PSP at both the control and different temperatures with a varied larval density has revealed that *D. yakuba* and *D. mauritiana* prefer to pupate maximum on the media and minimum on glass and cotton. The larvae of *D. rajasekari* prefer to pupate maximum on cotton and minimum on the media and glass. Whereas the larvae of *D. virilis*, *D. novamexicana*, and *D. hydei* prefer to pupate maximum on glass and minimum on the media. The larval PSP analyzed is affected by temperature and the larval density.

At lowest temperatures, the maximum pupation height occurs within *repleta* and *willistoni* groups and at higher temperatures all the species show a little pupation height or no upward movement (Schnebel, Grossfield, 1992). David and Clawel (1968), Srivastava and Singh (1998) reported the reduction of oviposition at low temperatures in different species of *Drosophila.*

Selection for faster development and that for adapting to larval crowding share some superficial similarity in the individuals failing to eclose before a certain point in time die, either because of food shortage, or because of being not included in the breeding adults. At high larval densities there is a clear environmental signal, in the form of food running out, available to the larvae such that they can make the switch from feeding to pupation. Rather than speeding up the development in real time, it is probably more important for larvae to acquire food faster than others. Such that they attain the critical size for pupation before their food runs out (Joshi, Mueller, 1996).

De Souza et al. (1968) reported that genetically determined pupation site choice in *D. willistoni* under a high density found that allelic variation at a single locus pupated in food cups or on the bottom of the population cage appeared to have an advantage and rapidly increased in frequency. Any larva pupating on the surface of the medium is likely to be buried if there is still an actively feeding larval population which suggests that the fitness of genotypes which vary in pupation height may be a function of larval density (Mueller, Sweet, 1986). Sokal et al. (1960) investigated those genetic and environmental factors that govern the selection of pupation sites by *D. melanogaster*. They reveal that densities below 52 eggs/vial do not affect pupation site, but at higher densities a greater proportion of larvae tends to pupate on the surface of the medium instead of on the wall of the vials. At the high-density level considered, there appears no relation between pupation site and density. In contrast, Pandey and Singh (1993), Sokolowski et al. (1997) and Joshi (1997) have reported that there is an increase in the pupation height with an increased larval density in *D. melanogaster*, *D.ananassae*, *D. bipectinata*, *D. malerkotliana*, and *D. biarmipes*.

The larval mortality varies from species to species at all the larval densities and temperatures. The highest larval mortality is found in *D. yakuba,* followed by *D. mauritiana*, *D. rajasekari*, *D. virilis*, *D. novamexicana*, and *D. hydei* compared to control (table 1). In a culture that contains a high density of larvae or adults, the food medium rapidly becomes very moist and soggy. In such cultures, individuals pupating on or close to the medium have an increased chance of being dislodged and drowned in the medium (Joshi, Mueller, 1993). Earlier studies also reported a highest pupal mortality at varying heights above the food surface on vials even at low densities (Joshi, Mueller, 1993; Borash et al., 1998).

The present study reveals that, compared to control, the percentage of media pupation decreased and glass pupation increased in *D. yakuba* with highest larval densities of 200 and 250 larvae/vial at lowest temperatures of 15 and 20°C (Figure 1 (1*b*)). Whereas the larvae of *D. mauritiana* increase the percentage of glass and media pupation with highest larval densities of 200 and 250 larvae/vial at lowest temperatures of 15 and 20°C (Figure 1 (2*b*, 2*c*)). *D. rajasekari* larval pupation on cotton decreases at 15°C and increases at 20°C at highest larval densities of 200 and 250 larvae/vial (Figure 1 (3*a*)). *D. virilis* larval preference on glass pupation decreases and the media pupation increases with highest larval densities of 200 and 250 larvae/vial at lowest temperatures of 15 and 20°C (Figure 2 (1*b*, 1*c*)). *D. novamexicana* larvae prefer glass for pupation more than the media with larval densities of 200 and 250 larvae/vial at lowest temperatures of 15 and 20°C (Figure 2 (2*b*, 2*c*)). In *D. hydei* larval pupation on glass decreases and media pupation increases at 15°C (Figure 2 (3*b*, 3*c*)).

At highest temperatures with lowest larval densities, *D. yakuba* larvae prefer the media for pupation at 100 and 150 larvae/vial at 30°C and decreases at 25°C. Whereas *D. mauritiana* prefers the media more and glass less for pupation at highest temperatures of 25 and 30°C at lowest larval densities 100 and 150 larvae/vial than the control. The larvae of *D. rajasekari* preferred to pupate maximum on cotton in control, wherein it decreased about 32 to 75.5% at highest temperatures of 25 and 30°C at lowest larval densities of 100 and 150 larvae/vial. In *D. virilis* the larval pupation on glass decreases and the media pupation increases at highest temperatures of 25 and 30°C in lowest larval densities of 100 and 150 larvae/vial. Whereas in *D. novamexicana* and *D. hydei* the glass pupation increases and the media pupation decreases at highest temperatures of 25 and 30°C at lowest larval densities of 100 and 150 larvae/vial. The combined effect of temperature and density at three different sites viz; cotton, glass, and media pupation analyzed by three-way ANOVA (temperature × density × sites) has revealed that the variation of larval pupation site preference in all the species is significant except *D. yakuba* on cotton, *D. rajasekari* on the media, and *D. virilis* and *D. novamexicana* on glass (table 2).

Barker (1971), Shirk et al. (1988), Shivanna et al. (1996), Vandal et al. (2003) reported that at constant temperature and density most of the species (*D. simulans*, *D. gibberosa*, *D. bipectinata*, *D. malerkotliana*, *D. yakuba*, *D. mauritiana*, *nasuta* subgroup species (*D. nasuta nasuta*, *D. nasuta albomicans*, *D. nasuta kepulauana*, *D. sulfurigaster sulfurigaster*, *D. sulfurigaster neonasuta*), *D. immigrans*, *D. rubida* and *D. pararubida*) preferred to pupate maximum on the media, *D. melanogaster*, *D. ananassae*, *D. virilis*, *D. novamexicana*, and *D. hydei* larvae preferred to pupate highest on glass, and *D. rajasekari* preferred to pupate highest on cotton. They have reported that the PSP depends on the quantity of glue protein synthesized by the salivary glands of the larvae. Comparison between all the species showed a variation in pupation with varying larval densities at different temperatures. The species collected from different geographical distributions and ecological backgrounds were maintained for about 2 decades at a constant temperature in the laboratory. They were not adapted for a constant temperature. When the larval densities and temperatures changed, the larval PSP also changes. This indicates that the larval density and temperature influences the PSP in different species of *Drosophila.* The temperature range is not similar for all the species. Differences in the temperature-dependent pupation responses among closely related species have been implicated as a possible basis for reducing interspecific competition (Fogleman, Markow, 1982; Ricci, Budnik, 1984; Schnebel, Grossfield, 1986; Kimura, 1988). The present study reveals that the significant variations in larval pupation site choices in sibling, sympatric, *virilis* and *repleta* group species are due to the extreme variations of temperatures and larval densities. Apart from the glue protein, locomotory path length, pattern and substrates, high and low temperatures and densities also affect the larval pupation site/habitat preference in *Drosophila*.

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