EFFECT OF TEMPERATURE AND DENSITY ON LARVAL PUPATION SITE PREFERENCE IN SIBLING SPECIES OF *DROSOPHILA* (DROSOPHILIDAE: DIPTERA)

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ABSTRACT: Larval pupation site preference (PSP) has been studied in sibling species of *D. melanogaster* and *D. simulans* at different density and temperature. It revealed significant variation between altered and controlled experiments. *D. melanogaster* larvae prefer to pupate less on glass and more in media when temperature and density altered than control. *D. simulans* larvae prefers glass lesser than control at 30°C and higher than control at 200 and 250 larvae/vial at 15, 20 and 25°C, whereas the media pupation is contrasts to the glass pupation in *D. simulans*. The media pupation is more at 30°C and less at other temperature at all type of density compared to control. *D. melanogaster* larvae does not prefer to pupate on cotton at 30°C, the larvae of *D. simulans* does not prefer o pupate on cotton at all density and temperatures. Two-way ANOVA revealed that variation of PSP in both the species at varied temperature and density at different sites was found to be significant.

KEY WORDS: *Drosophila*, pupation site preference, density, temperature, larva, habitat choice.

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 (Sameoto & Miller, 1968). The larval PSP has been analyzed by two types of phenotypic character one is the pupation height and the other is pupation site choice. The pupation height studies have been made using different factors such as moisture (Mensua, 1967; Sameoto & Miller, 1968), light (Pandey & Singh, 1993), temperature (Sokal et al., 1960; Schnebel & Grossfield, 1986; 1992), density (Singh & Pandey, 1993a), sex (Bauer, 1984), larval developmental time (Markow, 1979), selection for high and low pupation height and its genetic control (Casares & Carracedo, 1987; Singh & Pandey, 1993b). The larval PSP has also been studied by analyzing the percentage of pupae pupated at different sites viz; cotton, glass and medium in the culture. The studies of Barker (1971), Shirk et al. (1988), Shivanna et al. (1996) showed that under normal condition most of the species prefer to pupate maximum on media, and reported that the PSP has been related to the quantity of larval salivary gland protein. It is not influenced by increased water in the media and larval density, whereas the larval locomotory path length, pattern and substrates play a role in the preference of the sites for pupation (Vandal et al., 2003; Vandal & Shivanna, 2004; 2005a; 2005b).

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 increased egg size (Delpuech et al., 1995), larval and pupal duration (Gebhardt & Stearns, 1988; French et al., 1998), larval and adult size at eclosion (Gebhardt & Stearns, 1992; David et al., 1994), larger wing size (Noach et al., 1996; Imasheva et al., 1997), larval critical weight (de Moed et al., 1999;

Bochdanovits & De Jong, 2003), life span, life time fecundity and progeny production but reduces daily fecundity (Partridge et al., 1995).

Increased larval crowding of cultures results in a decrease of food availability over time and increase in metabolic waste levels especially ammonia (Borash et al., 1998). The major phenotypic effects of rearing larvae at high density (several hundred per vial) versus moderate (50 to 100 per vial) density has increased larval and pupal mortality (Joshi & Mueller, 1993; Roper et al., 1996), larval development time (Mueller et al., 1993; Chippindale et al., 1997; Prasad et al., 2001), pupation height (Joshi & Mueller, 1993; Pandey & Singh, 1993) and adult life span (Joshi & Mueller, 1997; Zwaan et al., 1991) whereas it reduced the adult size (Santos et al., 1997) and fecundity (Chippindale et al., 1993). De Souza et al. (1970) 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*. Larval adaptations are less specific than oviposition in response to temperature stress and it is more pronounced for puparia than adults in *Drosophila* (Kaneshiro et al., 1973; Coyene et al., 1983). Joshi (1997) reviewed the obvious effects of density on adaptations of both larva and adult, the food medium is rapidly becomes very moist and soggy, and individuals pupating on or close to the surface of the medium have an increased chance of being dislodged and drowned in the medium. The information about the effect of varied density in relation to temperature on larval PSP has not been available. In view of this it is planned to study the larval PSP using combined factors such as density and temperature. For the present investigation the sibling species, *D. melanogaster* and *D. simulans* belongs to *melanogaster* species group occupying different sites for pupation at constant conditions were taken.

MATERIALS AND METHODS

Sibling species, *D. melanogaster* and *D. simulans* were taken to study the effect of temperatures with varying larval densities on PSP (Bock & Wheeler, 1972; Ehrman, 1978). *D. melanogaster* and *D. simulans* primarily tropical but have expanded to temperate zones. These *Drosophila* species were collected from the *Drosophila* stock centre, University of Mysore, Mysore, India maintained since 20 years.

In order to maintain uniformity with regard to the density and age of the larvae the eggs were collected every 6 hours using modified technique of Delcour described by Ramachandra & Ranganath, (1988) and allowed to hatch. First instar larvae about 50 100, 150, 200, and 250 from the cultures were isolated and transferred to vial (10 X 3.8cm) containing equal quantity of wheat cream agar medium (Shivanna et al., 1996). About 50µl of dilute yeast was added everyday to feed the larva and to maintain the moisture content of the food medium. The culture vials with varied densities were kept at four different temperatures viz; 15C°, 20C°, (control 22 ± 1° C) 25C° and 30°C with RH 80%.

Ten replicates were carried out for each experiment. The mean values as well as percentage of pupation was calculated based on the number of larvae pupated at different sites viz; cotton, glass and medium. The primary data (number of pupae on different sites) was subjected to two-way ANOVA (SPSS software was used).

RESULTS

Table 1 shows the mean percentage of glass pupation at different larval density and temperature in *D. melanogaster* and *D. simulans*. It reveals that *D. melanogaster* prefer to pupate maximum on glass at different density and temperature. Compared to control, the glass pupation was decreased at all the temperatures and larval density except in 150 and 200 larvae/vial at 25°C. The larvae of *D. simulans* prefer to pupate minimum on glass compared to control; it was increased in 150 larvae/vial at 20°C and 25°C followed by 200 and 250 larvae/vial at 15°C, 20°C and 25°C respectively.

Table 2 reveals that *D. melanogaster* larvae prefer to pupate lesser on media than glass at different density and temperature. Compared to control the media pupation was increased in all larval densities and temperatures. The larvae of *D. simulans* prefer to pupate higher on media than glass at different density and temperature. Compared to control the media pupation was decreased in all larval density and temperatures except at 30°C in 100 to 250 larvae /vial.

Cotton pupation was found to be less at all temperature and density in *D. melanogaster*. The larvae of *D. melanogaster* do not prefer to pupate on cotton in control and 30°C. The cotton preference was increased in 250 larvae / vial at 15°C, 200 and 250 larvae / vial at 20°C and 25°C. The larvae of *D. simulans* do not prefer to pupate on cotton both in control and treatment experiment (Table 3).

The larval mortality was more in all density and temperature compared to control in *D.melanogaster* and similar in *D.simulans* except at 200 and 250 larvae/vial at 30°C and 250 larvae/vial at 25°C compared to control (Table 4). The combined effect of temperatures and densities at three different sites viz; cotton, glass and media pupation was analyzed by two-way ANOVA (temperature X density X sites), it revealed that the variation of larval pupation site preference in both the species is highly significant (Table 5).

DISCUSSION

In *Drosophila*, temperature is the most important environmental factor, which affects all biological process at the molecular, cellular and organismic levels (David et al., 1983). Temperature involves limitations on *Drosophila* behavioural alterations, which are expressed only at or above a critical temperature. Different species shows different optimum temperature for developing and some cannot be grown above certain temperature, the flies were moved to cooler place above 41°C (Grossfield, 1978). High temperature and dry periods for several days may act as strong selective force on developing pupae and the pupal survivorship decreases at lower temperature than the higher temperature in *D. melanica* (Tonzetich & Ward, 1972). *D. melanogaster* at control and different temperature with varied density preferred to pupate maximum on glass and minimum on media and cotton, whereas its sibling species *D. simulans* preferred to pupate maximum in/on media and minimum on glass.

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 genetic and environmental factors that govern the selection of pupation sites by *D. melanogaster*. It reveals that densities below 52 eggs / vial do not affect pupation site. At high-density level considered, there appears no relation between pupation

site and density (Sokal et al., 1960). In contrast, Pandey & Singh (1993) and Joshi (1997) reported that there is increase in pupation height with increased larval density in *D. melanogaster, D. ananassae, D. bipectinata, D. malerkotliana* and *D. biarmipes*. Present study reveals that percentage of glass pupation was decreased and media pupation was increased at all density and temperature in *D. melanogaster*, whereas *D. simulans* larvae showed contrasts results to *D. melanogaster*. Pupation on cotton was found at highest density and lowest temperature in *D. melanogaster*. Joshi & Mueller (1996) reported that at high larval density when there is a shortage of food to the larvae it switches from feeding to pupation.

Highest larval mortality was found in *D. melanogaster* at all density and temperatures in *D. simulans* highest mortality was observed with 150 larvae / vial at 15°C and 30°C, 200 and 250 larvae / vial at 20°C and 25°C compared to control (Table 4). In cultures of 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 surface of the medium have an increased chance of being dislodged and drowned in the medium (Joshi & Mueller, 1993). Earlier studies also reported highest pupal mortality above the food surface on vials even at low densities (Joshi & Mueller, 1993; Borash et al., 1998). Present study highest mortality was found at high temperature and density in *D. melanogaster* and *D. simulans*.

Vandal & Shivanna (2005a) showed no effect of water content of the medium and larval density on PSP in closely related sibling, sympatric, *virilis* and *repleta* group species when reared separately and reported that the (control) basic nature of PSP of the species has not been changed even though there is lack of space due to increased density. Among the species analyzed, *D. melanogaster* and *D. simulans* are closely related sibling species belonging to the *melanogaster* subgroup. It reveals that *D. melanogaster* and *D. simulans* prefer to pupate maximum extent on glass and medium at different density and temperature respectively. When the larvae were exposed to altered and combined temperature and density affected the PSP on all the sites in both the species. Two-way ANOVA revealed that variation of pupation site preference both in *D. melanogaster* and *D. simulans* at different sites with combined effect of density and temperature is found significant (Table 5).

The effect of larval density on PSP at constant temperature reveals that the larval PSP does not changed with increased density and they preferred to pupate as similar to control (Vandal & Shivanna, 2005a). The effect of temperature on larval PSP at constant larval density (50 larva / vial) revealed that at lowest temperature of 15°C the glass pupation was increased and decreased at highest temperature of 30°C (Vandal & Shivanna, 2007). The above studies indicate that density has no role in PSP whereas temperature plays major role in PSP. The present study reveals that when the larvae were exposed to both temperature and density simultaneously the PSP varies significantly. Therefore it is concluded that in presence of temperature, density also affects the PSP. Temperature and density together affect the larval behaviour, which causes the differential pupation site preference in sibling species of *Drosophila*.

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Table 1. Mean ± SD of glass PSP in different larval densities at different temperatures in *D. melanogaster* and *D. simulans* (Figures in parentheses are % of preference).

Species	15ºC	20ºC	25ºC	30ºC
D.melanogaster (Control, 94.2%)				
100 larvae/vial	82.2±4.2 (82.2)	87.1± 3.8 (87.1)	90.8±3.29 (90.8)	51.4±4.50 (51.4)
150 larvae/vial	125±3.88 (83.33)	111.7±3.59 (74.46)	141.7±3.56 (94.46)	91.1±3.78 (60.73)
200 larvae/vial	156.3±7.6 (78.15)	155.2±10.59 (77.6)	186.2±4.28 (94.6)	118.5±5.48 (59.25)
250 larvae/vial	192.2±5.7 (76.88)	216.9± 8.90 (86.4)	208.4±6.44 (83.36)	131.6±6.15 (52.76)
D.simulans (Control, 4.2%)				
100 larvae/vial	3.5±1.957 (3.2)	3.8±1.22 (3.8)	4.2±1.98 (4.2)	0.8±1.13 (0.8)
150 larvae/vial	4.5±1.509 (3.0)	4.3±1.49 (7.26)	7.2±1.31 (4.8)	6±0.96 (0.4)
200 larvae/vial	11.6±2.06 (5.8)	5.2±1.75 (12.6)	13.8±1.68 (6.9)	1.6±1.1 (0.8)
250 larvae/vial	27.1±7.68 (10.84)	10±2.90 (15.2)	21.4±2.5 (8.56)	2.1±1.59 (0.92)

Table 2. Mean ± SD media PSP in different larval densities at different temperatures in *D. melanogaster* and *D. simulans* (Figures in parentheses are % of preference).

Species	15ºC	20ºC	25ºC	30ºC
D.melanogaster (Control, 3.2%)				
100 larvae/vial	9.7± 2.9 (9.7)	7.5± 3.47 (7.5)	6.2±1.98 (6.2)	34.2±4.422 (34.2)
150 larvae/vial	13.4± 3.7 (9)	29.2± 2.69 (19.5)	5.4±2.01 (3.6)	47.4±3.06 (31.6)
200 larvae/vial	21.9± 4.0 (10.95)	37.5±9.32 (18.75)	7.7±2.11 (3.35)	62.4±7.39 (31.2)
250 larvae/vial	30± 2.8 (12)	18.7±5.35 (8.28)	31 ±7.27 (12.4)	94.0±5.51 (37.6)
D.simulans (Control, 93.2%)				
100 larvae/vial	92.3+2.311 (92.3)	91.3± 4.5 (91.3)	88.6±3.77 (88.6)	94± 1.69 (94)
150 larvae/vial	139.9+2.28 (84.66)	140.3±2.58 (88.93)	133.3±3.65 (88.86)	141.2± 2.29 (94.13)
200 larvae/vial	178.4+4.55 (89.2)	183±2.74 (81.5)	175.8±3.39 (86.35)	190.3±2.9 (95.15)
250 larvae/vial	208.7+6.11 (83.48)	226.4±3.77 (78.56)	218.4±4.69 (87.36)	240±2.9 (96)

Table 3. Mean ± SD of cotton PSP in different larval densities at different temperatures of *D. melanogaster* (Figures in parentheses are % of preference).

Species	15ºC	20ºC	25ºC	30ºC
D.melanogaster (Control, nil)				
100 larvae/vial	3.7± 2.1 (3.7)	----	----	----
150 larvae/vial	5.8± 1.9 (3.86)	----	----	----
200 larvae/vial	9.6±2.75 (4.8)	2±0.81 (0.76)	1.5±0.52 (0.75)	----
250 larvae/vial	14.9±3.1 (5.96)	3.8±1.22 (3.8)	2.9±1.37 (1.08)	----

Table 4. Percentage of larval mortality with varying larval densities at different temperatures in *D. melanogaster* and *D. simulans*.

Species	15ºC	20ºC	25ºC	30ºC
D.melanogaster (Control, 0%)				
100 larvae/vial	4.4	5.4	3.0	14.4
150 larvae/vial	3.81	7.04	1.94	7.67
200 larvae/vial	7.10	2.89	1.29	9.55
250 larvae/vial	11.12	5.32	4.24	9.64
D.simulans (Control, 4.2%)				
100 larvae/vial	4.5	4.9	7.2	5.2
150 larvae/vial	12.34	3.91	6.34	5.47
200 larvae/vial	5.0	5.9	6.75	4.05
250 larvae/vial	5.68	6.24	4.08	4.08

Table 5. Two-way analysis of variance of pupation site preference at three different sites in *Drosophila*.

Species	Source of variation	*F*- value
D.melanogaster		
	Temperature Vs Density (Glass)	137.81*
	Temperature Vs Density (Media)	13.23*
	Temperature VS Density (Cotton)	11.89*
D.simulans	Temperature VS Density (Glass)	29.15*
	Temperature Vs Density (Media)	1212.36*

$df_1=1$ $df_2=156$ * Significant at $P<0.001$ level