EFFECT OF UREA ON LARVAL PUPATION SITE PREFERENCE IN DIFFERENT SPECIES OF *DROSOPHILA*

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ABSTRACT: The effect of urea has been studied on larval pupation site preference (PSP) in different species of *Drosophila*, which occupy different sites for pupation. The larval PSP varies with different concentration of urea. The variation is found to be significant at different sites compared to control. The effect on viability varies in all the species. *D.simulans* larvae showed the maximum viability rate, which indicates the tolerance for urea, which are a nitrogenous waste and a toxin.

KEY WORDS: *Drosophila*, Larva, Pupation site preference, mortality, urea.

Urea is almost certainly a novel chemical for fruit flies since they do not produce it and are not likely to encounter it in their food: thus they should not have any preexisting adaptations to urea. Despite its toxicity, urea occurs naturally after mechanistic models to test in the urea adapted fruit flies. Effect of urea-alkali mixture on salivary glands of *Drosophila melanogaster* reveals that a "skeleton" exists in the discs of normal chromosomes which is resistant to nucleases but is destroyed by urea-alkali treatment (Kodani, 1942). In studies of interspecific competition between *Drosophila* species, the inhibitory effects of larval biotic residue on both larval viability and female fecundity were observed (Budnik & Brncic, 1974, 1976; Aiken & Gibo, 1979; Joshi et al., 1998; Borash et al., 2000). Subsequently, exposure to urea was shown to result in the slower development and reduced survivorship of *D. melanogaster* larvae (Botella et al., 1983, 1985; Joshi et al., 1996). Although it is not the primary metabolic waste of *Drosophila* larvae, urea has been shown to accumulate in *Drosophila* culture vials during the larval phase (Botella et al., 1985).

One vital aspect of the *Drosophila* life cycle is the ability of third instar larvae to pupate on substratum since puparia remains immobile and expose to potentially harmful environmental factors (Manning & Markow, 1981). 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 and dark (Pandey & Singh, 1993), temperature (Sokal et al., 1960; Grossfield, 1978; Schnebel & Grossfield, 1986, 1992; Pandey & Singh, 1993), 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 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) Vandal et al. (2003) 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 & Shivanna, 2004, 2005a,b). The information about the effect of urea on larval PSP has not been available. In view of this it was planned to study the effect of urea on larval pupation site preference.

MATERIALS AND METHODS

For the present investigation sibling species *D. melanogaster, D. simulans, D. yakuba* and *D. mauritiana* and sympatric species *D. ananassae, D. bipectinata* and *D. malerkotliana* (Bock & Wheeler, 1972; Ehrman, 1978; Grossfield, 1978; Singh & Pandey, 1991) were taken to study the effect of urea on larval PSP. These species 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 from the cultures were isolated and transferred to vial (10 X 3.8cm) containing equal quantity of wheat cream agar medium with different concentrations of urea (10%, 20%, 30% and 40%, SRL Lab grade) (Shivanna et al., 1996). One set of control was maintained without adding urea to the wheat cream agar medium. The culture vials were kept at constant temperature 22±1°C with RH 80% in BOD incubator. 50µl of yeast was added to the culture vials to feed the larvae everyday. Ten replicates were carried out for each concentration.

The larvae pupated at three different sites viz. cotton, glass and food medium were counted and recorded. The data was analyzed by statistical test to know whether the difference between sites, urea concentration and species are significant or not (two way-ANOVA).

RESULTS

Table 1 shows the percentage of media pupation in different species of *Drosophila* at different concentrations of urea. It reveals that the media pupation of *D. melanogaster* and *D. ananassae* was increased (about 12.2%, 23.4%, 21.2%, 14.8% and 48.8%, 35.6%, 34.4%, 33.8% in 10%, 20%, 30% and 40% of urea concentrations respectively) compared to control. The larvae of maximum media pupating species *D. simulans*, *D. yakuba* and *D. malerkotliana* decreases (about 91%, 88.4%, 91.4%, 89.0%; 14.8%, 15.2%, 6.4%, 6.6% and 41%, 63.4%, 69%, 72% in all concentrations of urea). *D. mauritiana* increases the media pupation about 57.4% and 55.4% in 10% and 40%, and decreases about 31.2% and 47.4% in 20% and 30% urea concentrations respectively compared to control.

Table 2 shows the percentage of glass pupation in different species of *Drosophila* to various concentrations of urea. It reveals that the maximum glass pupating species *D. melanogaster* and *D. ananassae* decreases (about 7.9%, 63.4%, 65.8%; 70.8% and 36.4%, 49.2%, 48.4%, 41.4% in all concentrations of urea respectively) whereas minimum glass pupating species *D. yakuba* larvae increases the glass pupation (about 73.4%, 74%, 83.2% and 80.2% in all concentrations of urea) compared to control. The glass pupation of *D. simulans* was increases (about 5.2% and 5.2% in 20% and 40% of urea) and decreases (2.4% and 2.2% in 10% and 30% of urea) and followed by *D. mauritiana* (20.6%,

20.2%, 16.8% and 7.2%) and *D. malerkotliana* (13.2%, 0.6%, 3.4% and 5.6%) decreases the glass pupation in all concentrations of urea compared to control.

Table 3 shows the percentage of cotton pupation in various species of *Drosophila* analyzed at different concentration of urea; *D. melanogaster, D. simulans* and *D. ananassae* do not preferred cotton for pupation. The cotton pupation of *D. yakuba* was decreased (0.2%, 1%, 0.2%, 1.6% in all concentrations of urea compared to control. The larvae of *D. mauritiana* increases the cotton pupation about 17.4% at 20% of urea and decreased in all other concentrations, whereas the larvae of *D. malerkotliana* increased the cotton pupation about 19.4%, 4.6% and 5 % in 10%, 30% and 40% except in 20% of urea compared to control.

Table 4 shows the percentage of larval viability and mortality in various concentrations of urea in different species of *Drosophila*. It reveals that *D. melanogaster* increases the rate of viability 91.2% in 10% of urea and *D. simulans* 94.2% in 40% of urea. *D. mauritiana* decreases the rate of viability 63% in 40% of urea and *D. malerkotliana* about 65% in 20% of urea. The percentage of larval mortality was found to be lowest in *D. melanogaster* about 8.8% with 10% of urea and *D. simulans* 5.8% to 6.6% in all the concentrations of urea whereas in other species the larval mortality rate was found to be highest in all the concentration of urea it ranges between 9.8% and 37% in *D. yakuba* and *D. mauritiana*.

ANOVA reveals that between different concentrations of urea the cotton pupation significantly differs in *D. yakuba* and *D. malerkotliana*. The glass pupation significantly differs in *D.mauritiana* and *D. malerkotliana* and the media pupation significantly differs in *D. melanogaster* and *D. malerkotliana* (Table 5). Two-way ANOVA between species v/s sites (glass and media) pupation is found to be highly significant ($F = 509.185$, $df_1=1$, $df_2=119$ and $F = 474.727$, $df_1 = 1$, $df_2=127$, $p<0.001$% level).

DISCUSSION

Studies of resistance to toxic chemicals, such as pesticides have usually considered compounds, which target a specific enzyme or biological process. Urea and ammonia differ from these in their wide-ranging effects on organismal and cellular physiology (Somero & Yancy, 1997). Earlier work has shown that *Drosophila* population can evolve resistance to high levels of environmental urea (Shiotsugu et al., 1997). Larvae in the urea adapted populations can developed into adults on food containing 300mmol-1 urea which normal larvae grow and pupate on this food but fail to eclose into adults (Shiotsugu et al., 1997; Joshi et al., 1998). In the present study, the larval pupation site preference varies from species to species with different concentrations of urea. It reveals that, the larvae of maximum glass pupating species *D. melanogaster* and *D. ananassae* increases the media pupation and decreases the glass pupation in all the concentrations of urea compared to control. Whereas the larvae of maximum media pupating species, *D. simulans, D. yakuba, D. mauritiana* and *D. malerkotliana* decreased the media and glass pupation in all the concentrations of urea compared to control.

In laboratory cultures the pupation site preference (PSP) of *Drosophila* varies from species to species, the larvae are seen to pupate in various proportions in the food medium, on the glass wall of the culture bottles and in the cotton used to plug the mouth of the culture bottle. These pupation sites where larvae pupate differ from one another with respect to surface structure and moisture content. Most of the studies regarding the effect of resources pH on pupation shows that

larvae pupated closer to the lowest pH resource than the highest pH resource and suggests that acidic resources affect the developmental time and the larva would travel greater distance to increase their fitness (Hodge et al., 1996). When using artificial resources of pH exerts clear effects on pupation height than the natural resource in *D. melanogaster* and *D. hydei* (Hodge & Caslaw, 1997). The effect of water on larval PSP reported that, the larvae of *D. melanogaster, D. ananassae, D. virilis, D. novamexicana* and *D. hydei* were moved from the culture and pupated on glass and *D. rajasekari* on cotton. Whereas *D. simulans, D. yakuba, D. mauritiana, D. bipectinata* and *D. malerkotliana* were do not moved from the media and pupated in/on media itself (Vandal & Shivanna, 2004). The present study reveals that, the media pupation of *D. melanogaster* and *D. ananassae* was increased whereas in *D. simulans*, *D. yakuba* and *D. malerkotliana* decreased and *D. mauritiana* showed both the characters with varied concentrations of urea. The glass pupation of *D. melanogaster*, *D. mauritiana*, *D. ananassae, D. malerkotliana* and *D. yakuba* was decreased. Whereas both character have been observed in *D. simulans*. The larvae of *D. mauritiana* and *D. malerkotliana* showed increased cotton pupation compared to control (Fig. 1).

In cultures, high levels of toxic nitrogenous metabolic wastes like urea, uric acid and ammonia are released to the media. The larval biotic residues are known to inhibit larval survivorship, female fecundity and adult size (Budnik & Brncic, 1974, 1976; Aiken & Gibo, 1979; Botella et al., 1983, 1985; Joshi et al., 1996). Relatively high levels of nitrogenous waste are likely being advantageous in crowded cultures of *Drosophila* (Joshi, 1993). Hodge (2001) reported that the mortality is due to high/low liquidity of the medium and the osmotic stress of the concentrated food resources. The present study reveals that *D. melanogaster* and *D. simulans* have increased the rate of viability (91.2% in 10% of urea and 94.2% in 40% of urea) whereas *D. mauritiana* and *D. malerkotliana* have decreased the rate of viability (63% in 40% of urea and 65% in 20% of urea). The percentage of larval mortality was found to be lowest in *D. melanogaster* and *D. simulans* in all the concentrations of urea whereas in other species the larval mortality rate was found to be highest in all the concentration of urea in *D. yakuba* and *D. mauritiana*. The effect on viability varies in all the species. *D. simulans* larvae showed the maximum viability rate, which indicates the tolerance for urea, which are a nitrogenous waste and a toxin.

Wilson (1997) and Srivastava et al. (2003), reported that some larvae failed to crawl from food on to the wall of vials for pupation but instead attempted for pupation on or near the surface of the food medium. They also suggested that, after treatment with cyromazine and penta co-ordinated organotin (IV) the larvae showed failure in wandering behavioural response in *Drosophila melanogaster*. Shivanna et al. (2006) have studied the effect of Endosulfan on larval pupation site preference in different species of *Drosophila*. They reported that the glass and cotton pupation has been decreased by the treatment of different concentration of Endosulfan in different species *Drosophila*. In the present study the effect of different concentration of Urea on larval pupation site preference in different species shows that, the media pupation was increased in maximum media pupating species whereas glass pupation was decreased and increased the media pupation in maximum glass pupating species compared to control even though they belongs to same species group.

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Table 1. Mean ±SD and percentage of media pupation in different species of *Drosophila* at different concentrations of urea.

Species	Concentration of urea				
	Control	10%	20%	30%	40%
D. melanogaster	1.6± 2.8 (3.2)	6.1±2.37 (12.2)	11.7±8.42 (23.4)	10.6±5.44 (21.2)	7.4±3.09 (14.8)
D. simulans	46.6±1.71 (93.2)	45.5±2.63 (91)	44.2±2.74 (88.4)	45.7±1.88 (91.4)	44.5±2.59 (89)
D. yakuba	29.3±1.94 (58.6)	7.4±3.30 (14.8)	7.6±2.50 (15.2)	3.2±1.75 (6.4)	3.3±1.88 (6.6)
D. mauritiana	25.7± 2.54 (51.4)	28.7±7.66 (57.4)	15.6±11.12 (31.2)	23.7±10.40 (47.4)	27.7±6.37 (55.4)
D. ananassae	4.6±2.59 (9.2)	24.4±7.96 (48.8)	17.8±3.19 (35.6)	17.2±2.89 (34.4)	16.9±6.31 (33.80)
D. malerkotliana	37.0±5.75 (74)	20.5±8.48 (41)	31.7±5.16 (63.4)	27.5±9.44 (69)	36±7.48 (72)

Table 2. Mean ±SD and percentage of glass pupation in different species of *Drosophila* at different concentrations of urea.

Species	Concentration of urea				
	Control	10%	20%	30%	40%
D. melanogaster	47.1 ± 4.37 (94.2)	39.5±4.97 (79)	31.7±8.08 (63.4)	32.9±6.83 (65.8)	35.4±5.18 (70.8)
D. simulans	2.3 ±1.159 (4.6)	1.2±2.29 (2.4)	2.6±2.01 (5.2)	1.1±1.96 (2.2)	2.6±2.87 (5.2)
D. yakuba	18.6±1.89 (37.2)	36.7±3.33 (73.4)	37.0±3.09 (74)	41.6±1.95 (83.2)	40.1±3.41 (80.2)
D. mauritiana	16.3±1.33 (32.4)	10.3±8.16 (20.6)	10.1±7.15 (20.2)	8.4±5.73 (16.8)	3.6±4.29 (7.2)
D. ananassae	40.7± 3.16 (81.4)	18.2±7.69 (36.4)	24.6±2.87 (49.2)	24.2±3.85 (48.4)	20.7±5.92 (41.4)
D. malerkotliana	8.4±4.71 (16.80)	6.6±5.42 (13.2)	0.3±0.67 (0.6)	5.8±5.26 (3.4)	2.8±3.08 (5.6)

Table 3. Mean ±SD and percentage of cotton pupation in different species of *Drosophila* at different concentrations of urea.

Species	Concentration of urea				
	Control	10%	20%	30%	40%
D. melanogaster	-	-	-	-	-
D. simulans	-	-	-	-	-
D. yakuba	1.4±0.97 (2.8)	0.1±0.31 (0.2)	0.5±1.08 (1)	0.1±0.31 (0.2)	0.8±0.78 (1.6)
D. mauritiana	0.7±1.87 (14.2)	0.7±1.15 (1.4)	8.7±9.73 (17.4)	1.8±2.34 (3.6)	0.2±0.42 (0.4)
D. ananassae	5.1±5.17 (10.20)	-	-	-	-
D. malerkotliana	1.8±4.39 (4)	9.7±7.58 (19.4)	0.5±0.70 (1)	5.4±7.36 (4.6)	2.5±1.77 (5)

Table 4: Percentage of larval viability and mortality in different species of *Drosophila* at various concentrations of urea.

Species	Different concentrations of urea							
	Viability				Mortality			
	10%	20%	30%	40%	10%	20%	30%	40%
D. melanogaster	91.2	86.8	87	85.6	8.8	13.2	13	14.4
D. simulans	93.4	93.6	93.6	94.2	6.6	6.4	6.4	5.8
D. yakuba	88.4	90.2	89.8	88.4	11.6	9.8	10.2	11.6
D. mauritiana	79.4	68.8	67.8	63	20.6	31.2	32.2	37.0
D. ananassae	85.2	84.8	82.8	75.2	14.8	15.2	17.2	24.8
D. malerkotliana	73.6	65	77.4	82.6	26.4	25.0	22.6	17.4

Table 5: ANOVA values for the effect of urea (10, 20, 30 and 40%) on larval PSP in different species of *Drosophila*.

Species	Cotton	Glass	Media
D. melanogaster	0.0	2.679	13.012*
D. simulans	0.0	1.931	1.654
D. yakuba	6.408*	2.058	1.105
D. mauritiana	0.157	10.213*	0.968
D. ananassae	0.0	0.628	1.371
D. malerkotliana	2.911*	10.083*	6.063*

$df_1=3$, $df_2=36$, * significant at $p<0.001\%$ level

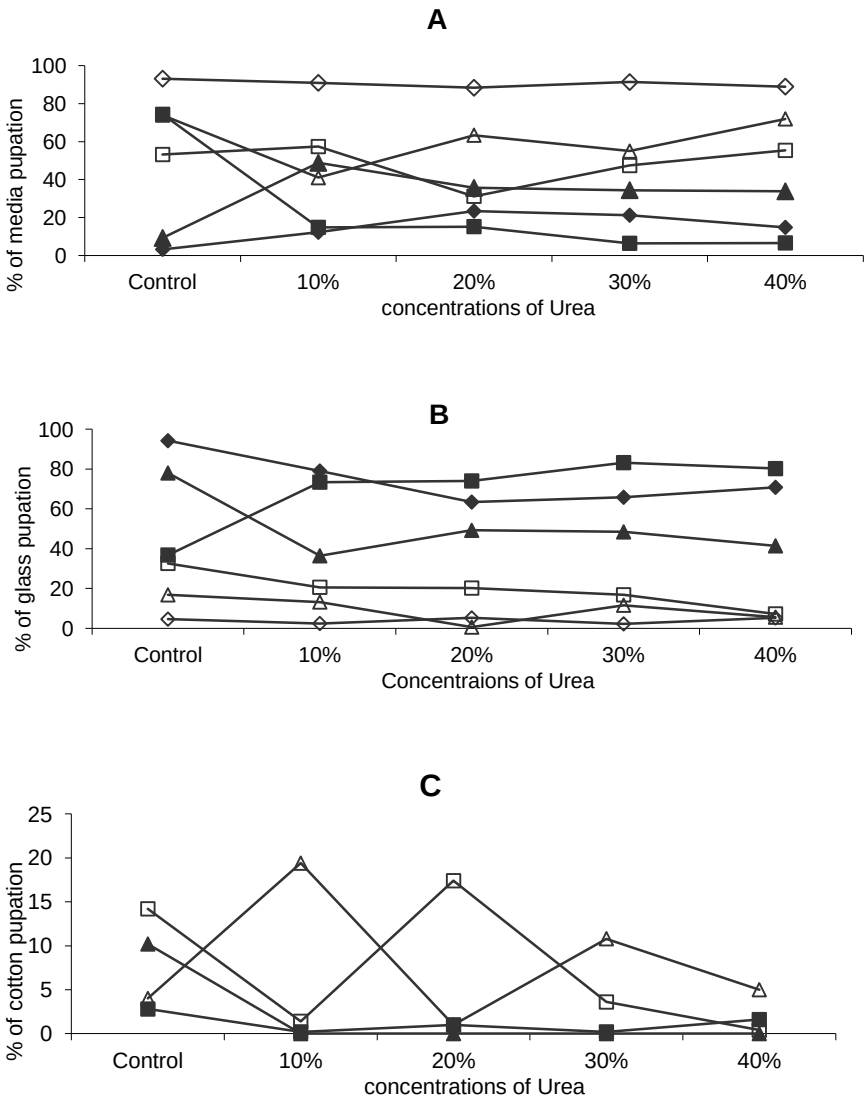

Figure 1: Effect of Urea on larval Pupation Site Preference in different species of *Drosophila*. A – Media, B – Glass, C – Cotton.

D.melanogaster *D.simulans*
D.yakuba *D.mauriatiana*
D.ananassae *D.malerkotliana*