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Zak, N.B. Experimental Medicine and Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel. A note on *tailup*.

tailup (tup) is one of six loci whose zygotic expression is required for germband retraction of the *Drosophila melanogaster* embryo. The *tup* locus has not been cloned. Two ethyl methanesulfonate-induced *tup* alleles have been isolated and the locus was determined to be in map position 54.0. It was cytologically placed between 37A1-B1 and 37B2-8 because it is removed by $Df(2L)137 = Df(2L)36C2-4;37B9-C1$ but not by $Df(2L)H68 = Df(2L)36B-C1;37A1-B1$ or $Df(2L)TW158 = Df(2L)37B2-8;37E2-F4$ (Nüsslein-Volhard *et al.*, 1984). Not surprisingly, we have observed that *tup* is removed by $Df(2L)TW3 = Df(2L)36F7-37A1;37B2-8$. We have tested three lethal loci, each representing one lethal complementation group that is uncovered by this deficiency, for allelism to *tup*. One of them, the ethyl methanesulfonate-induced mutation $l(2)37Aa$, is an additional *tup* allele. $l(2)37Aa$ is also known as $l(2)E41$, which was placed in the genetic location 53.1-53.9 (Wright *et al.*, 1976). $l(2)02660r$, a P element insertion allele generated by Paul Lasko at McGill University, falls within the *TW3* interval but is not allelic to *tup*. $l(2)02660r$ could serve as a good starting point for "local hopping" into the *tup* locus.

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Hodge, Simon^{1,3} and Paul Mitchell² 1. Dept. of Entomology and Animal Ecology, PO Box 84, Lincoln University, Canterbury, New Zealand; 2. Biology Division, Staffordshire University, College Road, Stoke-on-Trent, ST4 2DE, UK; 3. Author to whom correspondence should be addressed. The concentration of urea in the larval resource and its effect on larval performance.

Introduction: The excretion of metabolic wastes and secretion of enzymes for external digestion by dipteran larvae can alter the biochemical nature of their environment. This habitat modification can have both negative and positive effects on the success of other larvae which simultaneously or subsequently use the resource (Weisbrot, 1966; Dawood and Strickberger, 1969; Budnik and Brncic, 1975; Mitchell, 1988).

Urea has been identified as an excretory product of *Drosophila*, and at high concentrations has been shown to slow down the developmental rate of *Drosophila melanogaster* and reduce larval survival (Botella *et al.*, 1985).

This paper describes the amounts of urea produced by *Drosophila* larvae and re-examines the effects of urea on larval performance.

Methods: All experiments used wild-type stocks of *Drosophila*: 'Kaduna' for *D. melanogaster* and stocks reared from British flies for *D. hydei*. A temperature of 25°C, relative humidity of approximately 45% and a 16:8 hours light:dark regime was used in all cases. The experiments were carried out using standard glass vials (75mm x 25mm diameter) stoppered with foam bungs.

Vials of resource medium were prepared by hydrating 1.0g of ground Instant *Drosophila* Medium (IDM; Blades Biological Ltd., UK) with 4.0ml of distilled water. The vials of IDM were then seeded with three densities of first instar larvae: 0, 25 and 50. At least six replicates of each density were initially set up for both *D. melanogaster* and *D. hydei* (actual replicate numbers for each treatment for each particular assay are given in the Results section). The vials were left until the majority of the larvae had pupated and no larvae were visible in the resource; more specifically 8 days for *D. melanogaster* and 12 days for *D. hydei*. The remaining medium was then freeze-dried and stored at 4°C.

The above procedure was also carried out using 5.0g of mashed banana instead of IDM to examine urea concentrations produced when larvae were reared on a natural resource. The development of the larvae was slightly

elongated in this resource which meant that vials were left for a longer period: 9 days for *D. melanogaster* and 14 days for *D. hydei*.

Urea concentrations were determined using an assay formulated by Newell *et al.* (1967). This assay is extremely sensitive and can detect urea concentrations in the order of $20 \mu\text{g NI}^{-1}$. Samples of culture media to be used in the assay were reconstituted by combining one part freeze-dried resource and four parts distilled water (by mass).

Experiments were also carried out to assess the effect of urea on the performance of *Drosophila* larvae, and a series of urea solutions (0, 250, 500, 1000, 2000 μM) were prepared (this range encompasses the urea concentrations found in conditioned medium). 4.0ml of these solutions were used to hydrate 0.8g of IDM, and six replicates of each urea concentration were set up for each *Drosophila* species. Twenty five first instar larvae of either *D. melanogaster* or *D. hydei* were placed onto the surface of the medium after it had been hydrated. To examine whether urea in *Drosophila* medium could initiate a response at high concentrations, a 0.1M treatment was set up for *D. melanogaster* larvae only. Emerged adults were removed from vials every 24 hours and stored in 70% alcohol. The body size of the flies was estimated by a measure of female wing length, using the distance from the anterior cross vein to the wing tip along vein 3, with 10 specimens taken from each vial if available. The mean development time was calculated using all the emergent adults from each vial.

Results: The urea concentrations found varied extensively, and this is reflected by some large standard errors (Table 1). The data were analysed using the Kruskal-Wallis test. IDM and banana with *Drosophila* larvae present tended to have higher urea concentrations than the no-larvae controls. However, the only significant increase occurred in IDM conditioned by *D. melanogaster* larvae (Table 1).

Some 'urea' was found in the control media, where no larvae had been present. This may have been caused by interference with light absorption in the spectrophotometer due to pigments in the media or possibly some urea was produced by microorganisms in the media.

When examining larval performance, survival of *D. melanogaster* remained high for all treatments (Table 2) and was not significantly affected by the urea concentration of the medium. No difference in size of the female flies was found between urea treatments.

The mean development time of *D. melanogaster* larvae showed a significant response to urea concentration of the medium (Table 2); an elongation of the development time occurring when an excessively high urea concentration of 0.1M was used. This treatment was separated from the other urea concentrations using a Tukey test ($P < 0.05$).

No effect of urea concentration was found on *D. hydei* larval survivorship (ranging from 55-75%), or size of the emergent flies (Table 3). Mean development time of *D. hydei* showed a significant response to concentration of urea in

Table 1. Urea concentration (μM) found in medium (mean \pm SE(N)).

		Number of larvae			H	P
		0	25	50		
<i>D. hydei</i>	IDM	104.0 \pm 21.8 (6)	150.2 \pm 46.9 (6)	150.2 \pm 26.1 (6)	1.45	> 0.45
	Banana	74.0 \pm 36.1 (6)	125.8 \pm 26.6 (6)	123.8 \pm 19.6 (6)	3.32	> 0.15
<i>D. melanogaster</i>	IDM	55.0 \pm 11.8 (6)	352.2 \pm 71.1 (6)	230.7 \pm 26.0 (6)	13.5	< 0.001
	Banana	43.9 \pm 04.0 (6)	35.2 \pm 02.2 (4)	99.7 \pm 33.3 (3)	3.25	> 0.15

Table 2. Effect of urea concentration on *D. melanogaster* and larvae (mean \pm SE).

Urea Conc.	0	250 μM	500 μM	1000 μM	2000 μM	0.1M	F	P
Survival	95.2 \pm 3.0	98.8 \pm 1.3	96.0 \pm 2.1	89.2 \pm 6.0	96.0 \pm 3.3	90.0 \pm 4.1	0.9	> 0.45
Wing length	78.8 \pm 0.5	79.0 \pm 0.6	78.1 \pm 0.4	78.2 \pm 0.7	79.2 \pm 0.5	77.6 \pm 0.4	1.1	> 0.35
MDT	10.8 \pm 0.2	10.7 \pm 0.2	11.0 \pm 0.2	10.5 \pm 0.1	10.7 \pm 0.1	11.3 \pm 0.1	3.0	< 0.05

N = 6 (Survival - % larvae to adulthood; MDT, mean development time- days; wing length - graticule units 55 = 1 mm)

Table 3. Effect of urea concentration of *D. hydei* larvae (mean \pm SE).

Urea Conc.	0	250 μM	500 μM	1000 μM	2000 μM	F	P
Survival	72.0 \pm 3.6	56.8 \pm 7.1	69.2 \pm 9.4	75.3 \pm 5.6	68.8 \pm 3.6	1.1	> 0.35
Wing length	96.2 \pm 0.3	97.2 \pm 0.3	96.3 \pm 0.5	96.1 \pm 0.4	96.5 \pm 0.2	1.4	> 0.25
MDT	14.4 \pm 0.2	13.8 \pm 0.3	14.2 \pm 0.2	14.7 \pm 0.1	14.3 \pm 0.1	3.5	< 0.05

N = 6 (Survival - % larvae to adulthood; MDT, mean development time- days; wing length - graticule units 55 = 1 mm)

the medium (Table 3). This difference appears to arise from a probably-spurious reduction in development time when 250µM urea solution was used to hydrate the medium, although a Tukey test failed to isolate this development time from that found for other treatments.

Discussion: Urea has been previously identified as being produced by *Drosophila* larvae (Botella *et al.*, 1985) but the highest concentrations found in those experiments were higher, by about a factor of ten, than the concentrations of urea found in the current experiments. These higher concentrations of urea were probably caused by the higher densities of larvae used in those experiments (*c.f.*, 140 larvae/ml of medium with a maximum of 5/ml in this experiment) and that the experimenters utilised a method of 'larval stop', retaining larvae in the medium for an extended period. However, given these differences, the highest urea concentrations found in this study were still comparable with the lower values obtained by Botella *et al.* (1985).

Botella *et al.* (1985) suggested that urea could have a negative effect on the performance of *D. melanogaster*. However, the concentrations used to produce these responses seemed unrealistically high (0.03M to 0.2M) compared to the concentrations found in conditioned medium by those authors and in experiments carried out here. With the exception of the 0.1M treatment used for *D. melanogaster*, all the concentrations used in our experiment are below those of Botella *et al.* The negative response which *D. melanogaster* displayed to urea at the concentrations they used was linear for development time, a response occurring even at their lowest urea concentration (Botella *et al.*, 1985). The extension of the development time at 0.1M in this study is in accordance with those findings. Apart from the differences in urea concentrations used, there is another problem comparing the current work with that of Botella *et al.* caused by their method of 'larval stop' (Mensua and Moya, 1983). This produced larvae-to-adult development times of 23-35 days at 18°C (*c.f.*, 15 to 17 days for larval and pupal period of *D. melanogaster* at 18°C given in Ashburner and Thompson, 1978). It is possible that the effects of urea identified by Botella *et al.* (1985) are to be found only in the very specific conditions of their study.

It appears that urea can potentially have a deleterious effect on the performance of *Drosophila* larvae. However, these effects only occur at concentrations higher than those found even in contrived high density situations. It seems likely, therefore, that this substance would not occur at sufficiently high concentrations to affect *Drosophila* populations in nature.

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Norry, Fabian M.,* and Juan C. Vilardi. Laboratorio de Genética de Poblaciones, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires 1428 Buenos Aires, Argentina. Wing asymmetry and chromosome inversions in *Drosophila buzzatii*.

Abstract: The possible relationship between developmental stability and inversion karyotypes of the second chromosome was examined in the cactophilic fly *Drosophila buzzatii*. The results indicate that developmental stability, as indexed in terms of fluctuating asymmetry of wing length, does not differ among karyotypes in wild-reared flies. Thus, developmental stability is apparently independent of

both possible factors: (i) heterozygosity at the karyotypic level of variation, and (ii) any possible genetic coadaptation attributable to these chromosome inversions.

Introduction: Developmental homeostasis is the overall ability of individuals to cope with genetic and environmental stress (Lerner, 1954; Palmer and Strobeck, 1986; Parsons, 1990). In bilaterally symmetrical organisms, this ability may be indexed in terms of fluctuating asymmetry (FA) - side-wise random deviations from perfect bilateral symmetry (Van Valen, 1962). Inbreeding depression has often been thought to be causally associated with low levels of developmental stability (Lerner, 1954; Waddington, 1960, 1966). However, Fowler and Whitlock (1994) demonstrated that FA of sternopleural bristles is not a reliable measure of the degree of inbreeding in experimental populations of *Drosophila melanogaster*. Thus, although the FA level may be a reliable index of developmental stability, the genetic basis of FA remains unclear.

Two well-known hypotheses about the cause of genetic variation in developmental stability are heterozygosity and genetic coadaptation. While the isozyme heterozygosity has been examined with respect to FA in a wide variety of