Botella, L.M., A. Moya and J.L. Mensua. University of Valencia, Spain. Effect of urea on viability and mean developmental time of Drosophila melanogaster larvae.

By means of a series of experiments carried out with different strains of D. melanogaster, it has been observed--using an overfeeding technique (Mensua & Moya 1983)--that extreme competition gives rise to a developmental stop in third instar larvae. Two kinds of causes are both possible explanations of this delay in development: (1) waste products of larvae at high concentration could inhibit larval development by disturbing normal metabolism; (2) competition originates a scarcity of certain products necessary to further development (Budnik & Brncic 1976).

In order to test the first hypothesis, viabilities and mean developmental times were analyzed in non-competitive cultures (70 larvae in 5 ml of Lewis' medium) of an isogenic Oregon-R strain at different concentrations of urea, a waste product found in D. melanogaster cultures (Godbole et al. 1971). Experiments were carried out at 19±1°C. Table 1 shows data on viability and mean developmental time for seven different concentrations of urea and two controls without urea, one of these representing a highly competitive situation (70 larvae in 0.5 ml of Lewis' medium), and the other a non-competitive situation (70 larvae in 5 ml of Lewis' medium). Each datum represents the average of five replicae with standard error.

As can be seen from Table 1, viability does not decrease substantially up to the point where the level of urea reaches a concentration of 10 mgrm/ml. From this point onwards there is a drastic decrease in the number of emerged flies. On the other hand, mean developmental times increase, following a kind of function $y = ax^b$, as proved from the data, selecting the best fit among nine different functions of single independent variable (Table 2 and Figure 1).

The above data seem to indicate that some substances excreted by larvae into the culture medium can interfere with development, imitating overcrowding situations, as can be observed from Table 1, which compares viabilities and mean developmental times between the medium with high urea concentration (15 mgrm/ml) and the overcrowded control (70 larvae in 0.5 ml). Urea or similar waste products could act as developmental delayers, lengthening third instar as observed in jaw preparations. This situation leads either to death or to adult emergence. The way in which urea acts is different from that of other delayer substances such as formaldehyde (Sanmiguel and Rubio 1982) at 0.2% which produces developmental lengthening from the first larval instar: larvae are at the first or second instar at the time when they would be in the third instar in media supplemented with urea.

References: Bancroft, T.A. 1964, Biometrics 20:427-442. Budnik, M. and D. Brncic 1976, Evolution 29:777-781. Godbole, N.N., R.M. Kothari and V.G. Vaidya 1971, DIS 46:116. Mensua, J.L. and A. Moya 1983, Heredity 51:347-351; Sanmiguel, E. and J. Rubio 1982, XVIII Jornadas Luso-Españolas de Genética, Granada (Spain).

Table 1. Influence of different urea concentrations upon viability and mean developmental time in D. melanogaster.

Urea concentration (mgrm/ml)	Number of emerged flies	Mean developmental time (days)
0.0	62.8±2.0	24.3±0.2
0.5	64.6±1.6	23.9±0.3
1.0	64.4±1.0	23.4±0.1
2.0	62.2±1.1	24.1±0.2
4.0	61.8±2.3	26.9±0.1
8.0	59.6±1.6	27.8±0.3
10.0	56.4±1.6	28.5±0.6
15.0	21.6±8.9	30.5±0.7
0.0*	21.2±3.1	27.4±0.5

* overcrowded control

Table 2. Analysis of variance completed with regression of mean developmental times for different urea concentrations, averaged according to Bancroft (1964).

Source of variation	d.f.	S.S.	M.S.	F
Between concentrations	7	228.26	32.6	54.3**
Regression $y = ax^b$	1	220.43	220.43	237.0**
Deviation	6	6,83	1.3	2.2 n.s.
Within concentrations	32	19.04	0.6	
Total	39	475.56	254.33	

** $p < 0.001$ n.s. = non-significative

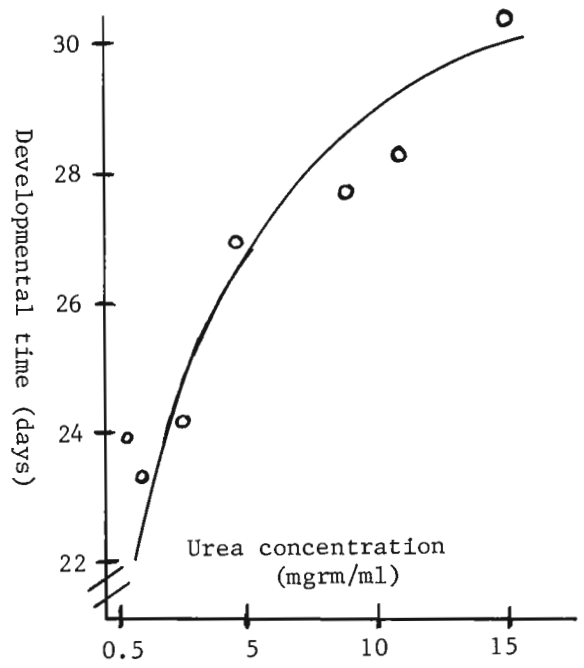

Figure 1. Mean developmental times for different concentrations of urea.

Best fit: $y = 23.086x^{0.096}$

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On the teratogenic effects of courmarin and hydroxycoumarin in D. melanogaster.

In a series of experiments, originally performed to establish LD_{50} values for Drosophila adults for a variety of drugs used in developing an in vitro teratogenesis assay (Bournias-Vardiabasis and Teplitz 1982; Bournias-Vardiabasis et al., in press), we observed that several of the surviving progeny which were exposed during oogenesis and larval period to these drugs showed various morphological defects. The majority of the drugs induced nonspecific defects such as unevaginated wings, deformed legs and fused abdominal segments. Only a small number (less than 1%) of the progeny were so affected.

Here we report defects found in progeny of females fed coumarin or hydroxycoumarin throughout oogenesis. The ensuing larvae also fed on the above named drugs both of which are known to act as teratogens in mammals (Shepard 1981) and in the Drosophila embryonic cell culture assay (Bournias-Vardiabasis et al. in press). Prior to this report there have been various workers reporting on phenocopy induction in Drosophila by heat shock (Mitchell et al. 1979) and various other agents (Ashburner and Bonner 1979) including some teratogens (Schuler, Harden and Niemeier 1982).

The feeding procedure was as follows: About 5-6 virgin Oregon-R females were placed in vials containing food plus the drug to be tested dissolved in food at the appropriate concentration. The females fed on the food for three days at which time all oocytes present would have been exposed to the drug for 72 hours since oogenesis in Drosophila takes that long to complete. The rational for exposing unfertilized eggs or oocytes to the drug through the mother is that once the egg is fertilized it is deposited by the females and then ceases to be under maternal control. The larva that emerges 24 hours later proceeds to

Table 1. Teratogenic effects of courmarin and hydroxycoumarin.

		Concentration			
			10^{-1}	10^{-2}	10^{-3}
A.	Coumarin				
	Number of adults scored	1250	0	158	1150
	Number of adults with defects	5	--	4	13
	% adults showing defects	0.4	--	2.5	1.1
B.	Hydroxycoumarin				
	Number of adults scored	--	0	712	1356
	Number of adults with defects	--	--	15	16
	% adults showing defects	--	--	2.0	1.2