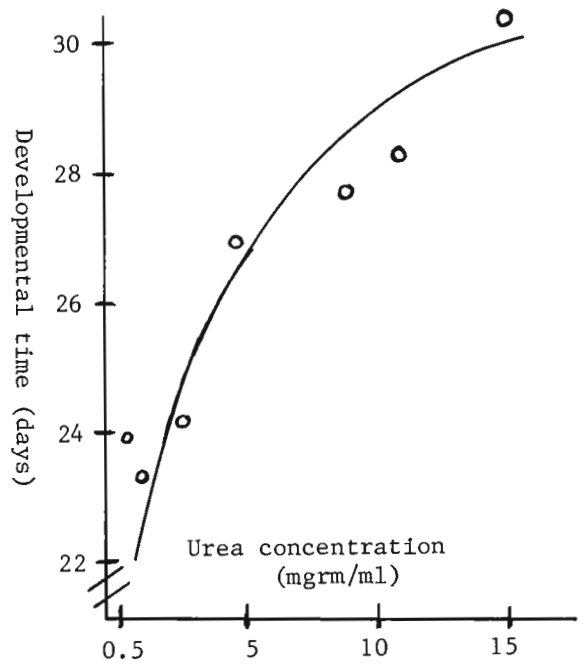

Figure 1. Mean developmental times for different concentrations of urea.

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

feed until pupation takes place and then the adult emerges. Thus, under this protocol the ensuing progeny have been exposed to the drug indirectly during oogenesis (through the maternal circulation) and during the larval period by ingesting the drug directly. Both coumarin and hydroxycoumarin were tested at 10^{-1}, 10^{-2} and 10^{-3} M.

Exposure of Drosophila to hydroxycoumarin or coumarin resulted in greater than 1% of progeny showing defects. A dose-dependent response was also observed with both drugs (Table 1). The defects observed included missing eye facets and various wing malformations.

The results indicate that both coumarin and hydroxycoumarin act as teratogens in Drosophila. The defects we have observed indicate that extensive cell death in the imaginal wing disc must have occurred. In the case of the eye-antennal disc, only specific areas must have been affected. To investigate this further, we plan to stain late third instar discs with a vital stain and compare drug-treated ones for areas of cell death with controls. With further experimentation (drugs and dosage) this protocol might serve as a useful teratogen screen. A further refinement of the protocol will be to include techniques to alter egg permeability (Limbourg and Zalokar 1973) to allow for exposure of fertilized eggs during the first 24 hours when embryogenesis takes place.

References: Ashburner, M. and J.J. Bonner 1979, Cell 17:241; Bournias-Vardiabasis, N., R.L. Teplitz, G.F. Chernoff and R.L. Seecof (in press), Teratology; Bournias-Vardiabasis, N. and R.L. Teplitz 1982, Terat. Carcin. Mut. 2:333; Limbourg, B. and M. Zalokar 1973, Dev. Biol. 35:382; Mitchell, H.K., G. Miller, N.S. Petersen and L. Lipps-Sarmiento 1979, Dev. Genet. 1:181; Schuler, R.L., B.D. Harden and R.W. Niemeier 1982, Terat. Carcin. Mut. 2:293; Shepard, T.H. 1981, in: Catalog of Teratogenic Agents, John Hopkins Press, Baltimore.

Castro, J.A., A. Moya and J.L. Mensua. University of Valencia, Spain. Gene frequency-dependent selection: Analysis of competition among two and three competitors of Drosophila melanogaster.

One of the methods employed to detect gene frequency-dependent selection is the so called ratio diagrams (Ayala 1971; Anxolabéhère 1980; Wallace 1981). This procedure has been used not only to analyze competition between two species but also between two different genotypes of the same species. It has recently been applied to competition situations among three genotypes by Tosić and Ayala(1981).

The results obtained from experiments with two and three competitors of D. melanogaster in highly competitive situations (72 larvae in 0.5 ml of Lewis' medium) were analyzed by this method. The strains used were the following: a wild stock (wild), and two eye-colour mutants (cardinal, cd III-75.7 and sepia, se III-26.0). In the cultures with two competitors three possible competition situations were taken into account: wild/cd, wild/se and cd/se. The genetic composition of each system was 68/4, 64/8, 56/16, 36/36, 16/56, 8/64 and 4/68. In the case of three competitors, the following genotype compositions were studied:

wild	64	4	4	40	16	16	32	20	20	28	28	16	24	8	32	32
cd	4	64	4	16	40	16	20	32	20	28	16	28	24	32	8	32
se	4	4	64	16	16	40	20	20	32	16	28	28	24	32	32	8.

In order to apply the ratio diagrams method in the case of three competitors, the following ratios were selected: 64/4+4, 40/16+16, 32/20+20, 28/28+16, 24/24+24, 16/28+28, 8/32+32, 20/20+32 and 4/4+64. In both cases (two or three competitors) a total of fifteen replicae were made for each genetic composition.

Figure 1 shows the ratio diagrams for two competing genotypes. Figure 2 shows the same for the three competing genotypes (wild/cd+se, cd/wild+se and se/wild+cd). The analysis of regression was carried out using the mean values of the repetitions. Thus, the t-test gives a greater reliability to the fit by using a lower number of degrees of freedom. Reliability is lower when the repetitions are considered as independent experiments.

When we consider the competition between cd/wild, the slope of linear regression is significantly smaller than one. We can then assume a negative gene frequency-dependent selection, and that when the frequencies of cd are very low a stable equilibrium point is reached. On the other hand the ratio diagrams of se/wild reflects a constant selectivity against se. The same occurs with se/cd where cd displaces se.

In competition among three genotypes the wild/cd+se ratio diagram shows a slope significantly smaller than one, meaning a negative gene frequency-dependent selection with a point