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.


One of the methods employed to detect gene frequency-dependent selection is the so called ratio diagrams (Ayala 1971; Amxolabhêtre 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, 36/36, 16/36, 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 replicates 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
Figure 1. Ratio diagrams for the regression of the output ratio to the input ratio in the two-genotype experiments at high density. The values plotted are the logarithms of the ratios.

Figure 2. Ratio diagrams for the regression of the output ratio to the input ratio in the three-genotype experiments at high density. The values plotted are the logarithms of the ratios.

of stable equilibrium at very low frequencies of $cd+se$. In the $cd/wild+se$ ratio diagram the slope significantly approaches the 0.05 level, with a stable point of equilibrium being set. In the case of the $se/wild+cd$ the displacement of $se$ is kept, with constant selective responses.

Therefore it would seem (between two competitors as well as among three competitors) that $se$ is always displaced in competition with wild and $cd$, being a bad competitor and following a frequency-independent behaviour. As a consequence of the results from wild and $cd$ strains with one or two competitors, stable coexistence between both strains can be admitted when $cd$ is found at very low frequencies.