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The development of resistance to various organophosphates in populations of D. melanogaster.

Among the main problems associated with the use of insecticides is the development of resistance to particular chemicals and cross resistance to a variety of other chemicals conferred by just one. Over fifty economically and socially important species of insects and mites are now known which show some resistance to one or more organophosphorous compounds. In only a few of these cases has a mechanims of resistance been investigated, but in most of them it is a gene with primarily dominant effects which is responsible.

We have now obtained populations of D. melanogaster which are resistant, either singly or multiply, to six different organophosphates. These range from the simple to the complex and from the relatively nontoxic to the highly toxic. These populations are shown in Tables 1 and 2.

Those populations in Table 1 were started on 18th May, 1972, from a stock of Ore R which had been rendered lethal-free by the Cy BlL technique. The populations in Table 2 were started on 6th February, 1971, as F_1 hybrids between Ore K and Ore R stocks.

The populations are kept in standard metal population cages with 16 food pots which are changed every 2-3 weeks. The phosphates are added to the food medium during the last stages of food preparation. In those populations with two or more phosphates applied, each phosphate is added singly to between 4-8 of the food pots. We have not, as yet, done any experiments in which more than one phosphate is present in all the media. The concentrations are increased when visual inspection shows a sufficient number of larvae emerge from food medium containing the obtained dose to suggest a degree of resistance is present.

Table 1. Resistance of populations of D. melanogaster to organophosphates applied singly.

Compound	Normal toxic level	Now resistant to
TEP	0.018M	0.1M
TEP	0.018M	0.13M
TMP	0.02M	0.04M
TBP	0.02M	0.11M
TIPP	0.004M	0.02M
DEMETON-S-METHYL	10ppm	100ppm
DDVP	0.1ppm	0.8ppm

Table 2. Resistance of populations of D. melanogaster to organophosphates applied multiply.

TEP	0.018M	0.1M
TMP	0.02M	0.06M
TEP	0.018M	0.1M
TBP	0.02M	0.07M
TEP	0.018M	0.1M
TIPP	0.004M	0.03M
TEP	0.018M	0.1M
TMP	0.02M	0.06M
TBP	0.02M	0.05M
TEP	0.018M	0.1M
TIPP	0.004M	0.02M
TMP	0.02M	0.05M
TBP	0.02M	0.05M

TMP - Trimethylphosphate
TEP - Triethylphosphate
TBP - Tributylphosphate
TIPP - Triisopropylphosphite
DDVP - Dimethyl 2, 2 dichlorovinylphosphate

These populations are now available for investigation but one or two points are worth further comment now. Firstly, we have shown in experiments with samples from these populations that there is a measure of cross resistance between a number of these compounds. Resistance to TMP confers resistance to TEP and vice versa, for example, although not very efficiently. More important, perhaps, is the fact that TEP, at least, confers a considerable degree of resistance to DDVP. We have demonstrated this using WHO type aerosol tests on samples from our populations.

In those populations in which TMP only is applied, the maximum dose we have been able to reach is 0.04M. In those in which TEP and TBP are also applied it is 0.06. The difference is due to the male sterilising activity of TMP at doses above 0.002M (Dyer and Hanna, 1972). This sterility is not present in males developing in TEP media which we have mentioned, does confer TMP resistance.

Preliminary tests on the mechanism of TEP resistance by means of diallele crosses among

the various resistant populations suggests that most of the resistance is contributed by the effects of one or a small number of almost completely dominant genes.

Reference: Dyer, K.F. and P.J. Hanna 1972, Mutation Res. 16:327-331.

Garcia-Bellido, A. and J. Dapena. Centro de Investigaciones Biologicas, C.S.I.C., Madrid, Spain. Recovery of cell marker mutants in Drosophila.

This is a preliminary report on some new cell marker mutants detected by mitotic recombination in somatic cells. Wild type males were mutagenized, with EMS (0.3%), and crossed for three subsequent generations with their sisters. The males of the third generation were then crossed to cn bw;e females, their offspring were irradiated (1.000R X-rays) as 84 ± 12 hrs. old larvae to induce mitotic recombination (MR) and the adult males were singly mated to SM5/ $T(2,3)$ e/Ubx^{130};C(4)RM spa^{pol} females. Once their offspring were ensured, the males were sacrificed and one by one mounted for microscopic examination. Their dorsal thoraces and abdomens were then scored for the appearance of clones of abnormal cuticular structures. The treated chromosomes of the presumptive mutant-carrying males were kept balanced over SM5; Ubx^{130}. Identification of the mutant-carrying autosomes and arm localization was again carried out by mitotic recombination. Quantitative analysis of twin spots with known cell marker mutants (Garcia-Bellido, 1972 Mol. Gen. Genetics 115:54 -) gave the approximate mitotic location in the chromosome. In order to locate them meiotically the balanced stocks were crossed to a standard multiply marked (MM) chromosome and the F_1 daughters were backcrossed to the same MM stock. Their offspring were irradiated and the different crossover classes studied for the presence of MR spots of the cell marker mutant. Since this mutant could simultaneously be accompanied by some induced lethals, a viability test of the cell marker mutant was carried out by crossing the complementary left and right recombinant classes carrying the mutant.

As to the usefulness of the new mutants found, a ranking (RK 1-3) has been attempted which states the penetrance of the marker in different organs and cuticular structures. For the sake of comparison mwh is ranked RK 1.

The present method allows the detection of induced - or spontaneous - cell differentiation mutants located anywhere in the Drosophila genome already in heterozygous flies, independently of whether the mutant or the chromosome is a zygotic lethal.

pawn (pwn, 2:58.3 between pr and c); in 2R, 52.2% of the MR between centromere and sdp). Affects both chaetes and trichomes. Chaetes appear truncate due to the depigmentation and subsequent loss of the tip. Trichomes are pin-shaped with a thin transparent process. Homozygous poorly viable in zygote. RK1 in abdomen, RK1 for trichomes and chaetes in the thorax.

sandpaper (sdp, 2:83.1 between pr and px; in 2R, 62.6% of the MR between centromere and y($Dp\ sc^{S2}$). Affects only the pigmentation and the trichome pattern on the tergites (RK1). Cuticle depigmented, trichomes super-numerary, thick, densely packed and uncombed. Lethal - or associated with lethal - in homozygotes. Non-detectable in the thorax.

flare (flr, 3:38.8 between h and th; in 3L, 69% of the MR between centromere and jv). Affects both chaetes and trichomes. The former have a rudimentary socket and their shaft is frequently crooked and branched. Trichomes are transformed into multiple short outgrowths over the entire cell surface. Lethal - or closely associated with a lethal - in homozygotes. An allele of flare, non complementing for lethality, has been independently found. RK1 in both thorax and abdomen. Affects cell viability in thorax.

bald (bld, 3:48.1 between st and cu; in 3R, 87.5% of the MR between centromere and Ki). Affects chaetes, trichomes and cuticle pigmentation. Cuticle completely depigmented, which manifests in transparent chaetes and thin, wooly trichomes. Lethal - or associated with lethal - in homozygotes. RK1 in both thorax and abdomen.

comet (cmt, 3:57.2 between cu and sr; in 3R, bld is 44.5% of the MR between centromere and cmt). Affects chaetes and trichomes. Chaetes small and thin. Trichomes similar to mwh but less regular and with a lower number of processes per cell. Lethal - or associated with lethal - in homozygotes. RK3 in abdomen, for trichomes in thorax RK1.