Korochkin, L.I. and N.M. Matveeva.
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Novosibirsk, USSR. Esterase isozymes of Drosophila virilis group.

Some workers have demonstrated the existence of multiple forms of esterases in Drosophila virilis (Sims, 1965; McReynolds, 1967; Ohba & Sasaki, 1968). Esterase patterns have been studied in a number of D. virilis and D. texana stocks obtained from the collection of Prof. N. Sokoloff (Institute of Developmental Biology, Moscow). The starch and polyacrylamide gel procedures were used to analyse about 1000 adult individuals of D. virilis 101 (wild type), 147 (b bk dt), 140 (va), 103 (R,gl), 142 (w) and of D. texana 123 (wild type), 119 (dt) (the numbers correspond to the stocks designations used in the collection). The esterases were detected using α-naphthylpropionate as substrate (yielding best results) or a mixture of α- and β-naphthylacetate as substrates. Two types of electropherograms are distinguished: the "virilis" type and the "texana" type (Fig. 1). The zymograms of different D. virilis stocks show 6 esterase groups all of which display interstock polymorphism and some intrastock polymorphism. In adult flies the slowest esterase 1 exhibits weak activity and is represented by three bands (a,b,c). Esterase-2 in 140 and 142 stocks is stained very intensely, in stock 147 the staining is somewhat weaker and in stock 103 the staining is faint in most cases. In the incubation mixture of α- and β-naphthylacetate this esterase preferentially acts on naphthylacetate and unlike all the other D. virilis esterases is stained red. Stocks 140, 101, 142 are characterized by a slow variant of esterase-2 (B), and stocks 103 and 147 by a fast variant (A). Esterase-3 stains more intensely in males; however, sex polymorphism was clear-cut only on days 4-5 after outflow. Esterase-4 (consisting usually of two bands one deep staining and the other minor) is the second "main" esterase in the electropherogram with very high activity in D. virilis. Stocks 140 and 101 show a typical "fast" form of esterase-4 (A), stock 147 a "slow" form (B), whereas in stock 103 and particularly 142, both forms are present as homozygotes as well as heterozygotes. Esterases-5 and 6 stain less intensely in most stocks excluding 103 which differs from all the other stocks in that its activity is more evenly distributed among all the different esterase fractions.

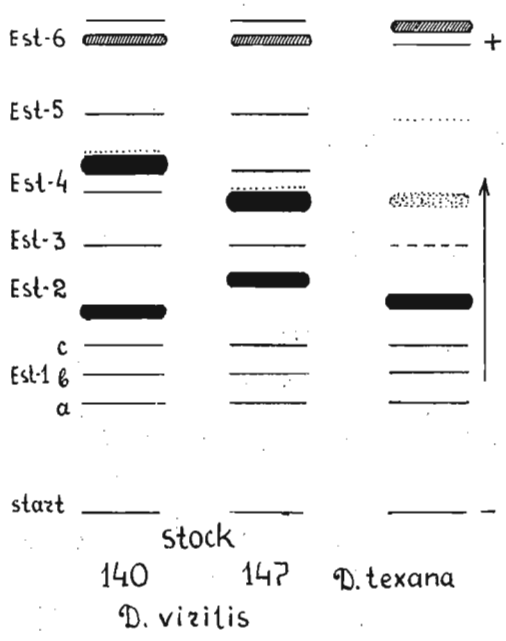

Fig. 1. Electropherograms (starch gel)

Table 1

Treatment	Esterases 1	2	3	4	5	6
Specificity to substrate	α	β	α	α	α	α
10^{-3}M $CuSo_4$	±	-	±	-	-	-
10^{-5}M E-600	+	±	±	±	++	-
10^{-4}M physostigmine	+	+	++	+	+	+
Heating 50° 10 min	±	+	±	±	±	±
Heating 60° 10 min	±	++	±	+	+	++
Heating 70° 10 min	++	++	++	+	+	++

(++) - complete inhibition
(+) - weaker reaction
(±) - indefinite or variable results
(-) - no effect

D. texana stocks 123 and 119 have identical esterase patterns which differ from the pattern of D. virilis in that, first, esterases 4 and 5 are, as a rule, very indistinct, with esterase-5 usually being unidentifiable and second, in addition to esterase-2, esterase-b and esterase-6 also display an affinity for β-naphthylacetate. Table 1 shows the affinity of esterases of D. virilis stock 101 for inhibitors. D. virilis ♀ 140 x ♂ 147 and ♀ 147 x ♂ 140 esterase 4 hybrids are represented by two fractions, a paternal and maternal fraction, and esterase-2 is composed of three fractions - two parental and hybrid. Experiments with dissociation-reassociation have shown that esterase-2 is a dimer.

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Mutagenic effect of azathioprine in Drosophila melanogaster.

Azathioprine is a drug extensively used for immuno-suppression and the treatment of neoplasias. Since many of the patients treated with azathioprine are of reproductive age, it is important to evaluate the potential genetic risks associated with the use of the drug. Preliminary mutagenicity studies were carried out with D. melanogaster.

Canton-S males were treated for 24 hours by feeding on 10mg. azathioprine spread on the surface of the usual treacle/semolina medium (10 flies/vial). Treated males were mated to 3 virgin Canton-S females every 3 days, to give a total of 5 broods. The percentage of unhatched eggs was used as an indicator of dominant lethality. The results obtained are shown in Table 1.

Table 1.

	Brood 1		Brood 2		Brood 3		Brood 4		Brood 5	
	% u.h.	No.	% u.h.	No.	% u.h.	No.	% u.h.	No.	% u.h.	No.
Control	13.8	3675	13.8	3824	15.1	3438	15.0	2980	13.2	2173
Treated	15.3	3473	19.1	3627	19.2	3350	19.1	2993	15.8	2305
χ^2	3.1		24.3		20.1		17.8		6.0	

u.h. - unhatched eggs

The ability of azathioprine to cause loss or breakage of chromosomes was tested by treating C(1)RM, y/R(1)2, vf/B^S Y y^+ males as above, and mating to virgin y w^a females with 3 day brood intervals for a total of 4 broods. The progeny were scored for sex, loss of Y^L, loss of Y^S, loss of X or Y and non-disjunction. The results are presented in Table II. The effect of azathioprine on the sex-ratio in rod-X flies was tested by treating C(1)RM, y/y v/B^S Y y^+ males and mating as above.

Table 2.

Series	Total progeny	Male/Female	Complete loss X or Y, %	Loss Y^L, %	Loss Y^S, %	N.D.J. %
Control	14,212	0.863	0.197	0.030	0.060	0.118
Brood 1	8,264	0.908	0.096	0.076	0.076	0.162
2	7,080	0.999	0.141	0.085	0.113	0.198
3	4,847	1.005	0.289	0.247	0.206	0.165
4	2,904	0.971	0.482	0.140	0.070	0.475

Azathioprine induced dominant lethals in Canton-S males in post-meiotic and meiotic broods. In the treated ring-X stock, chromosome breakage, as indicated by partial chromosome loss, is highest in brood 3 (χ^2 = 7.10 for loss of Y^L). In brood 4, the maximum frequency of complete X or Y chromosome loss occurs (χ^2 = 6.89), and this coincides with the highest frequency of non-disjunction (χ^2 = 3.38), indicating that meiotic germ cells are being utilized in this brood. However, it is noted that although peak chromosome loss and non-disjunction coincide in brood 4, the non-disjunction does not account for all the chromosome loss. There was no evidence that preferential loss of the Y^L or Y^S occurred.

Only 4 mosaics were observed amongst 95 exceptional progeny in the treated series, and were subsequently found to be fertile. Only one exchange event was found. Of the 27 progeny with partial chromosome losses in the treated series, 5 were fertile, 2 of which were mosaics. Marker loss in 3 flies was not accompanied by loss of fertility factors.

In brood 3, the ring-X sex-ratio differed significantly from that of its control (χ^2 = 21.12). The rod-X, male/female, sex-ratio was greatest at brood 3, and did not differ significantly from its control (χ^2 = 0.23). From this it can be inferred that much of the sex-ratio shift observed in the progeny of treated ring-X flies can be attributed to breakage of the ring chromosome.

This study demonstrates the genetic activity of azathioprine in D. melanogaster. Tests in mammalian systems are required to evaluate further the mutagenicity of this drug.

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(Moscow) for providing them with D. virilis and D. texana stocks.

References: McReynolds, M. 1967, Genetics 56:527-540; Ohba, S. and F. Sasaki 1968, Proc. Twelfth Internat. Cong. Genetics, Tokyo:156-157; Sims, M. 1965, Nature 207:757-758.