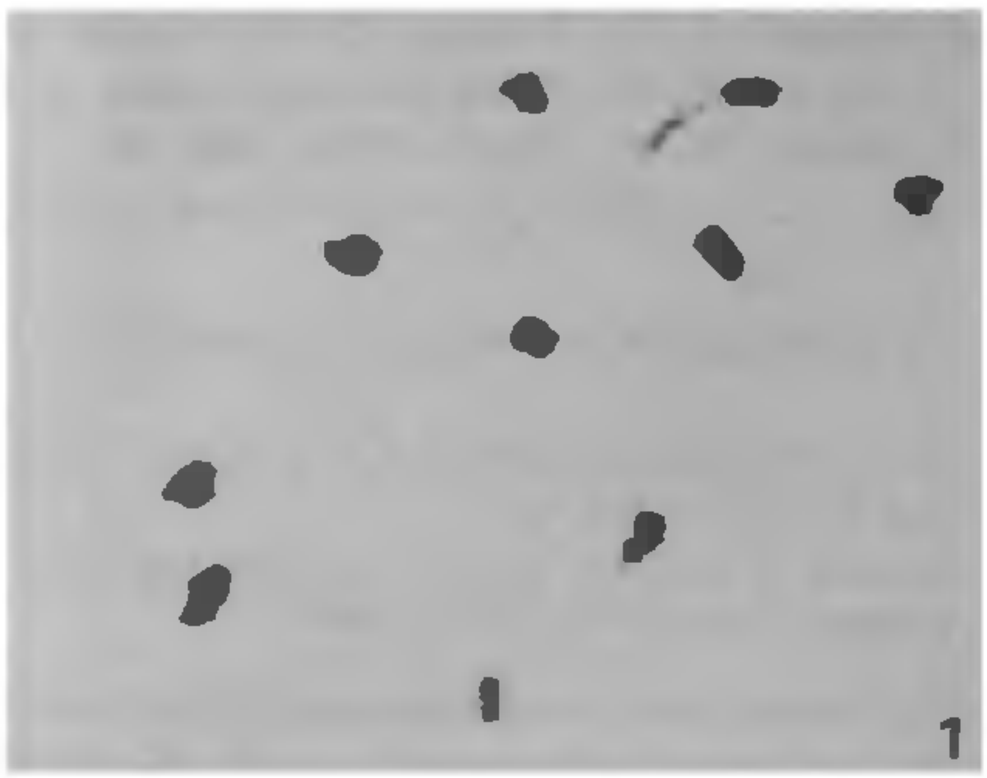

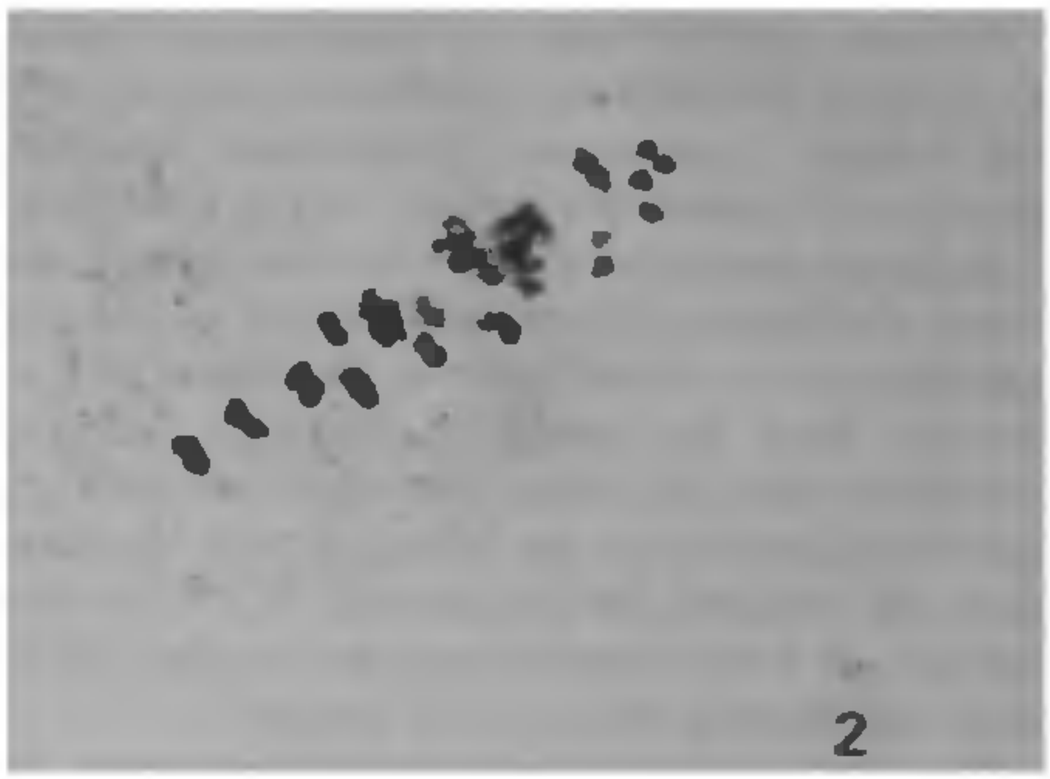

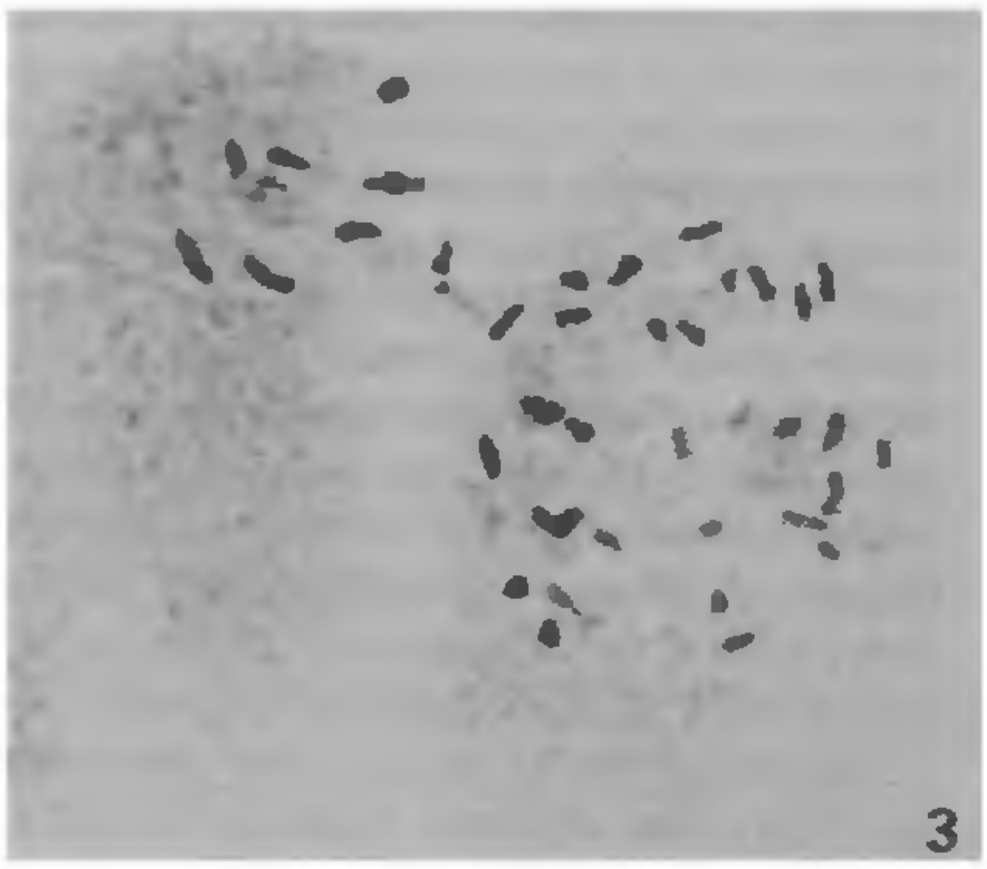

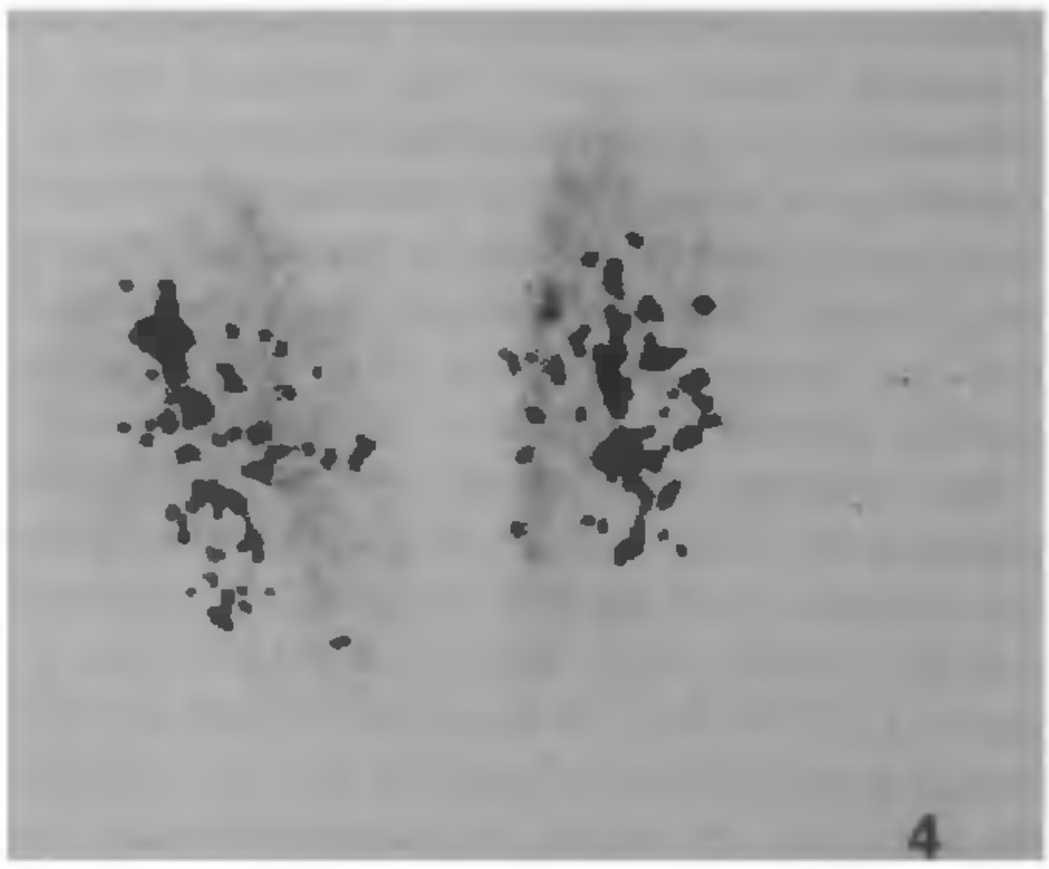

Figures 1–4. 1. Diakinesis in normal cell of trisomic 5 of maize showing 1 III + 9 II; **2.** Metaphase I with double the number of chromosomes; **3.** Chromatin material from one cell passes to another, and **4.** Dyad with many chromosome fragments.

different stages of meiosis, from early prophase to tetrads, in the same anther, was similar to that observed by Levan[2] in haploids of *Phleum pratense*.

In triploids and its F_1 descendents including trisomics of maize, McClintock[5] observed double the number of chromosomes at metaphase I, which was presumed to be the result of fusion of nuclei in the early prophase or even premeiotic fusion. Several anaphase I cells with chromosome fragmentation were also observed. But the chromosomes which are in trisomic condition in plants showing cytomixis were not identified in that study[5].

Aneuploidy and the imbalance caused by the extra chromosome in the trisomic may be a factor responsible for the cytomixis observed in trisomic 5 of maize and the chromosome fragmentation may be an after effect of cytomixis.

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GENOTOXICITY OF DORMIN-5 IN DROSOPHILA SEX-LINKED RECESSIVE LETHAL AND FEMALE GERM LINE MOSAIC TESTS

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SINCE the discovery of benzodiazepines in 1950's, their use as sedative-hypnotic, antianxiety, anti-

convulsant and muscle relaxant has extensively increased. Reports of their abuse in varying degrees are available[1]. Several tranquilizers are known to inhibit RNA synthesis, mitosis and induce chromosome aberrations[2–6]. Mutagenic actions of tranquilizers have been established in *Drosophila melanogaster*[7,8]. Reports concerning the mutagenicity of nitrazepam are scanty[9]. Gupta *et al*[10], however, have reported this compound to be nonmutagenic in *Salmonella* reversion assay. The present note describes the genotoxicity of Dormin-5 (nitrazepam, manufactured by the Centaur Laboratories Pvt. Ltd., Bombay) grouped as benzodiazepine under the head minor tranquilizers, through the sex-linked recessive lethal (SLRL) test and the female germ line mosaic test in *D. melanogaster*.

Flies to be tested came from a cross between w^{co}/w^{co} females and $fs(1)K_{10}w$/Y males. $fs(1)K_{10}$ (= K_{10}), a sex-linked female sterile mutation (1–0.5), which causes abnormal egg shape[11], is used as the marker for female germ line mosaicism. The standard Muller-5 (*Basc*) technique[12] was followed for detecting the sex-linked recessive lethals induced by the tranquilizer.

Eggs were collected from the above cross for 4 h on standard Drosophila food. Larvae, after 24 h and 72 h, counted from the middle of the egg collection period, roughly corresponding to the beginning of the 1st and 3rd larval stages respectively, were collected by floating them in 50% glycerol. Nearly 100 larvae of each instar (in several replicates) were exposed to different concentrations of the tranquilizer, powdered and thoroughly mixed in the food. The LD_{50} for each larval instar was determined as the concentration where roughly 50% of the larvae developed to adult stage. Thus the LD_{50} for the 24 h larvae was 0.01% and for the 72 h larvae it was 0.06% (w/w). The larvae were fed with the LD_{50} and one half of such doses for the rest of their larval life[13]. For SLRL test, individual w^{co}/Y males, fed as larvae on normal and Dormin-5 supplemented food, were crossed with 3 *Basc* homozygous females for 3 days. Each of the resulting $F_1 w^{co}/Basc$ females was sibmated with the *Basc*/Y males and the presence or absence of w^{co}/Y males in the F_2 generation was noted. For the female germ line mosaicism, 24-hour-old individual K_{10} heterozygous females, fed as larvae on normal and Dormin-5 containing food, were allowed to lay eggs in plastic vials on normal food darkened with charcoal powder[14]. Eggs were collected and counted every day for 10 days and the number of K_{10} eggs laid was recorded. The average daily egg production was calculated by dividing the total number of eggs laid by the total number of K_{10} heterozygous females tested and the number of days of egg collection. All the experiments were conducted at 25 ± 1°C. The SLRL data were statistically analysed using the Kastenbaum and Bowman test[15] and the female germ line mosaicism data with the χ^2 test.

The results of the SLRL test (table 1) show a significant increase in the number of sex-linked recessive lethals in all the treatments except the 24 h larvae exposed to 50% of the LD_{50}. One male developing from the 24 h larvae and 2 males developing from the 72 h larvae respectively, exposed to the LD_{50}, yielded 2 lethal chromosomes each indicating clusters[16,17]. Calculations on the basis of Poisson distribution followed by a χ^2 goodness of fit[18] indicated no significant difference between the expected and observed frequencies ($P > 0.05$) which suggests that the lethals originated from independent events and do not represent clusters. The SLRL test is regarded as the best validated mutagenicity test in *Drosophila*. It is argued[19] that such lethals originate from gene mutation, deletions of small chromosome parts and certain types of chromosome aberrations. Since, in

Table 1 *Induction of sex-linked recessive lethals after Dormin-5 exposure of Drosophila larvae*

Larval age	Conc. (%)	Males tested	Chromosomes tested		Concl.*	Lethal chromosomes/male		
			Total	Lethal (%)		0	1	2
Control	0.00	197	3200	0.16		192	5	0
24 h	0.01	63	820	1.34	+	52	9	1
	0.005	49	667	0.60	–	45	4	0
72 h	0.06	60	791	2.15	+	48	8	2
	0.03	64	997	1.30	+	51	13	0

* Conclusion on the basis of Kastenbaum and Bowman test[15]; level of significance $P < 0.01$.

Table 2 *Induction of female germ line mosaicism after Dormin-5 exposure of Drosophila larvae*

Larval age	Conc. (%)	Females tested	Mosaic (%)	Concl.*	Total eggs laid	% K_{10} eggs	Average daily egg production
Control	0.00	258	1.16		54,402	0 02	21.05
24 h	0.01	102	10.78	+	20,306	0 09	19.91
	0 005	125	7.20	+"	18,704	0.06	14.96
72 h	0.06	109	15.60	+	19,632	0 11	18 01
	0 03	110	11.82	+	19,250	0 11	17.50

* Conclusion on the basis of χ^2 test; " level of significance $P<0.05$; level of significance $P<0.001$.

both mouse bone marrow cells and *Allium* root tip cells, nitrazepam was reported to induce chromosome and chromatid type breaks[9], the induction of sex-linked recessive lethals was expected.

The data on female germ line mosaic test are given in table 2. The frequency of mosaic induction is significantly higher in all the treatments. However, the average daily egg production slightly decreased in the treated series. This assay is based on the exposure of K_{10} heterozygous larvae to a chemical mutagen and the mosaic females are recognized as the ones laying K_{10} eggs occasionally[14]. The progeny of cells homozygous for K_{10} in the female germ line will form K_{10} eggs. A mitotic recombination in the female germ line cells or a mutation in the K_{10}^{+} gene or its loss through chromosome breakage can lead to K_{10} homozygosity[20]. Since nitrazepam is reported to induce chromosome and chromatid type breaks[9] and since DNA-double strand breaks are a prerequisite for mitotic recombination[21], the induction of K_{10} homozygosity in the female germ line of *Drosophila* in our experiments may be related to the action of this tranquilizer. Thus a significant increase in the frequency of mosaic females in the different treated series, irrespective of the exact mechanism involved, indicates that Dormin-5 is genotoxic to the female germ line cells of *D. melanogaster* in the toxic range.

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