Effect of intercalating mutagens on crossing-over in *Drosophila melanogaster* females

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Summary. In order to evaluate the effect of several intercalating compounds on crossing-over in *Drosophila melanogaster* females, acridine orange, acriflavine, chloroquine, ethidium bromide and quinacrine were fed separately to larvae of *y ct f*/+ + + genotype. Our results show that acridine orange, acriflavine and ethidium bromide increase significantly the recombination frequency at the *ct-f* region and support the view that, for intercalating agents, there is a relationship between clastogenic activity and female recombination induction.

Key words. Drosophila melanogaster; intercalating mutagens; crossing-over; clastogenic activity; recombination induction.

Crossing-over is a mutual exchange of parts of chromatids of homologous chromosomes. It takes usually place during chromosome pairing at meiosis, but occurs in some organisms also at mitosis. In *D. melanogaster*, meiotic recombination normally occurs only in females and it is absent in the male sex. However, since the first studies of Hiraizumi[1], several authors have confirmed the occurrence of spontaneous male recombination in *D. melanogaster*, albeit at frequencies much lower than in females[2]. The frequency of meiotic crossing-over in *D. melanogaster* females (and males) can be increased by many factors such as high temperature, X-rays and several chemical mutagens.

Intercalating agents are chemicals, generally planar heterocyclic compounds, that bind electrostatically between adjacent DNA base pairs[3]. This binding process can produce varied and multiple biological effects, including mutagenic effects. In a previous paper[4], we reported that acridine orange and ethidium bromide, two typical intercalating agents, are able to increase significantly the crossover values in males of *D. melanogaster*. This finding together with the results of Suzuki[5] who found that actinomycin D (another intercalating compound) increases the frequency of meiotic recombination in females, caused us to perform the present experiments in order to test the ability of several other intercalating agents to increase meiotic crossing-over in *D. melanogaster* females.

Materials and methods. 1) Strains. Two strains of *D. melanogaster* were used for this study: Berlin-K (BK), a wild-type stock maintained for a long time in the laboratory, and *y ct f*, a stock marked with 3 X-chromosomal recessive mutants, *y* (yellow, 1–0.0), *ct* (cut, 1–20.0) and *f* (forked, 1–56.7). 2) Chemicals. Acridine orange (AO), acriflavine (AF), chloroquine (ClQ) and quinacrine (Q) were obtained from Sigma. Ethidium bromide (EB) and ethyl methanesulfonate (EMS) were obtained from Merck. All chemicals were diluted in an aqueous solution which contained 5% sucrose (Merck). For concentrations see table. 3) Procedures. Eggs were collected by crossing 3–4-day-old *y ct f* virgin females with BK males of the same age in special cages[4]. Samples of 100 eggs were seeded in bottles with 25 ml of standard food medium enriched with living yeast. 24 h later, when the bottles contained 1st-instar larvae, 1 ml of test solution was added to each bottle. Two controls were made in parallel: the normal control group was treated with 5% sucrose only and the positive controls were treated with solutions of EMS, a well-known alkylating mutagenic agent that increases the frequency of crossing-over in *D. melanogaster*[6]. The F_1 offspring was intercrossed and the F_2 progeny was counted and classified in order to estimate the frequency of recombinant flies. All the experiments were performed at 25 ± 1 °C.

Results and discussion. The results of the crossing-over experiments are presented in the table. The statistical procedure used was a chi-square test. As can be seen, only 2 (AF and EB) of the 5 intercalating agents tested produced a significant increase in the total crossing-over frequency at both concentrations employed. With EMS, used as positive control, a significant increase in the total crossover frequency was found too but only at the higher concentration.

It is interesting that, with the exception of 0.5% AF, the increase in total crossover frequencies is mainly due to an increase in the *ct-f* region, which is close to the centromere. This is in agreement with results obtained by other authors with different chemicals[6–8].

Crossing-over is known to be induced in oogonia, and probably in stem cells of *D. melanogaster* females, by radiation, mitomycin C, actinomycin D, nitrosoethylurea and caffeine, and it was found that somatic crossing-over in *D. melanogaster* occurs primarily in proximal heterochromatin[9,10]. Thus, a plausible explanation for our results could be that the increase in the recombination frequency in the *ct-f* region is due to induction of mitotic recombination in the germinal line.

It is further assumed that the ability of a chemical to induce recombination depends on its clastogenic activity. Clastogenic agents, such as mitomycin C and X-rays, increase significantly

Frequencies of recombination in the *y-ct* and *ct-f* regions respectively after treatment of *D. melanogaster* larvae with several intercalating mutagens

Compound tested	Concentration	Non recombinant progeny	Recombinants Crossover regions *y-ct*	%	*ct-f*	%	Total	%
Control	–	3498	995	15.76	2040	32.31	2816	44.60
EB	1 mM	2055	674	16.59	1454	35.79[c]	2008	49.42[c]
	3 mM	2410	758	16.53	1564	34.11[b]	2175	47.44[c]
AF	0.1 %	2492	705	14.98	1651	35.07[c]	2215	47.06[b]
	0.5 %	1577	558	18.57[c]	971	32.31	1428	47.52[b]
AO	0.2 mM	2090	646	16.62	1322	34.02[a]	1796	46.22
	0.5 mM	2342	649	14.96	1471	33.91[a]	1995	45.99
Q	5 mM	2315	585	14.25	1325	32.27	1791	43.62
	10 mM	2360	684	15.68	1447	33.17	2002	45.90
ClQ	5 mM	1515	446	16.15	891	32.26	1247	45.15
	10 mM	2240	666	16.31	1321	32.36	1842	45.12
EMS	3 mM	2086	608	15.73	1325	34.28[b]	1779	46.03
	6 mM	2119	701	16.70	1543	36.76[c]	2079	49.53[c]

[a] Significant at 0.05 level, [b] significant at 0.01 level, [c] significant at 0.001 level (based on chi-square test).

the recombination frequency in females of *D. melanogaster*[8]. If this supposition is general, we can expect that intercalating agents with clastogenic activity must increase the recombination frequency, as was found for actinomycin D[5].

AF, AO and EB, which significantly increase the crossover frequency in the *ct-f* region in this experiment, act as clastogenic agents in *D. melanogaster*[11,12], while ClQ and Q, which have no effect on recombination frequency, do not seem to exert clastogenic effects either. From our results it appears also that there is a relationship between clastogenic effectiveness and the capacity to induce female recombination. Given the fact that some intercalating agents increase female recombination significantly, and others do not, it is difficult to explain this only on the basis of the induced changes in the DNA conformation. According to Filipski's hypothesis[13], intercalating compounds may competitively inhibit the repair reaction of some nick-closing enzymes and the inhibition of nick repair may be the actual cause for the increase of recombination frequencies.

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Radiation-induced hyperdiploidy in *Hyoscyamus niger* L.[1]

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Summary. A hyperdiploid plant type, approaching the triploid chromosome number, and representing possibly a high level of tetrasomy, was recorded in the progeny of a gamma ray-induced unbranched desynaptic mutant in the M_4 generation. Its meiotic behavior and its possible importance for deriving diverse hyperdiploid lines from desynaptic mutants are outlined.
Key words. Hyperdiploid plant; *Hyoscyamus niger;* radiation-induced mutant.

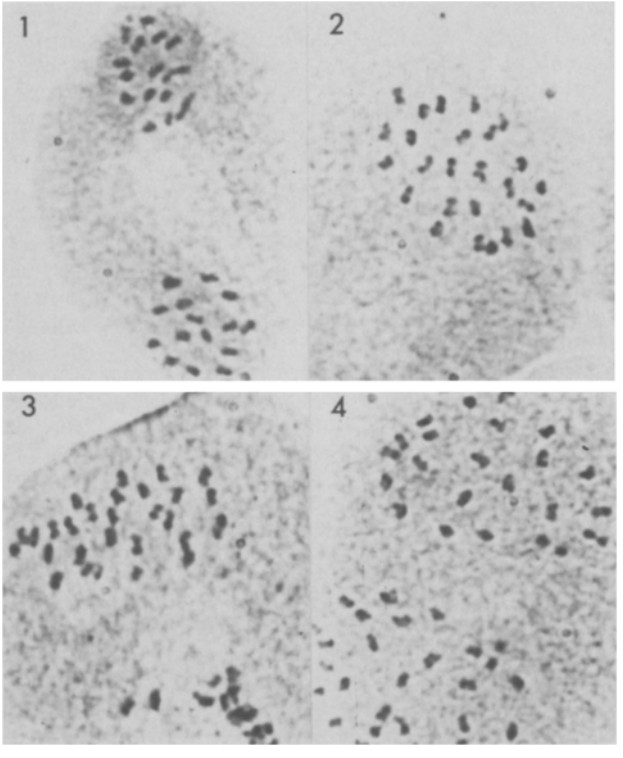
Sharma and Singh[2] reported an unbranched tall mutant in *Hyoscyamus niger*, a solanaceous plant of immense therapeutic value, after 20 kr gamma irradiation of seeds. Its productivity and progeny performance were evaluated. It was found that this mutant which had a high potential for various agronomic characters (plant height, number and size of leaves, etc.) and crude drug content, was a poor seed setter. The analysis of meiotic behavior revealed this mutant to be weakly desynaptic which in turn caused a wide array of symmetric and asymmetric anaphase disjunctional patterns[3]. Incidently, in the M_3 and M_4 populations of this mutant, certain plants did not set seeds either on selfing or in reciprocal crosses with normal control plants. Meiotic analysis was performed on such a plant in M_4, which revealed its hyperdiploid nature, the details of which are given below.

Material and methods. Flower buds of appropriate size were collected between 09.00 and 10.00 h in Carnoy's fixative for meiotic analysis from the sterile mutant plant (MTU-11). This plant resembled the other mutant plants in all characters except fertility. Meiotic and pollen preparations were made following the usual staining in 2% acetocarmine. At least three anthers from each of five flower buds were examined.

Observations. The male meiosis in this plant revealed nearly a triploid chromosome number (diploid 2n = 34) with chromosome numbers varying between 48 and 54, and depicting an array of anaphase I distributional patterns (table, figs 1–4).

At diplotene/diakinesis, metaphase I, the occurrence of a few tetravalents was also noted (i.e. 2–6 tetravalents per cell, the exact frequency distribution of which could not be recorded as there was not sufficient material available from these stages). The pollen grains revealed complete sterility as discerned by nonstainability by acetocarmine.

Discussion. The mutant in question was found to be fully sterile. This sterility is possibly caused by an added dose of chromo-



Figures 1–4. Chromosome separation at anaphase I in *H. niger*. *1* 17:17 separation in control, *2–4* anaphase I in hyperploid plant, *2* one of the anaphase I poles showing 25 chromosomes, *3* 27 chromosomes on one pole and the other pole with chromosomes in two groups, a sign of disturbed polarity, *4* 23:28 separation.