Technical Papers

Occurrence of Mitotic Crossing-over Without Meiotic Crossing-over

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The occurrence of crossing-over has been demonstrated not only during the meiotic cycle but also during mitotic multiplication of cells in certain plants and animals (*2, 6–9*). The purpose of the experiment to be reported here is to see if the gene c3G in *Drosophila melanogaster* which nearly eliminates meiotic crossing-over also suppresses mitotic crossing-over.

C3G is a recessive gene in the third chromosome (*5*). Externally it produces no visible effect in either homozygous or heterozygous constitution. However, it eliminates nearly all meiotic crossing-over in the first, second, and third chromosomes and causes frequent nondisjunction of chromosomes I and IV as well as the production of intersexes, supersexes, triploids, and numerous sterile eggs. It presumably acts by nearly eliminating meiotic pairing in the female, the nonsynapsed chromosomes being distributed at random without having undergone crossing-over (*3*).

Mitotic crossing-over can be studied by means of mosaic spots due to crossing-over in somatic cells ("somatic crossing-over"). The latter will result in groups of cells homozygous for genes carried initially in heterozygous condition in the developing zygote. Genes in the X-chromosome were chosen for the present study, and females carrying recessive sex-linked genes in heterozygous constitution were obtained. The genes had to be suitable to produce observable effects in small patches of cells on the surface of flies whenever the cells had become homozygous due to crossing-over. Genes meeting these requirements are y (yellow body color, 1, 0.0) and sn^3 (singed bristles, 1, 21.0). In order to obtain flies homozygous for c3G and heterozygous for the genes y and sn^3, two stocks were employed: (1) homozygous for the normal alleles of y and sn^3 and also homozygous for c3G (++;c3G), and (2) homozygous for y sn^3 and also homozygous for c3G (y sn^3;c3G). For the latter stock we are indebted to Miss Eva Rosenow (Mrs. Fred Sherwood). Flies of both stocks were crossed reciprocally, and the F_1 female offspring (y sn^3/++; c3G/c3G) were examined for the occurrence of mosaic spots. Since crossing-over occurs at the four-strand stage in somatic cells as well as in meiosis, yellow singed spots indicate crossing-over between the kinetochore and the sn locus, yellow spots result from crossing-over between the y and sn loci, and singed spots from double crossovers.

As a check on the ability of the observer to identify spots a control cross of y sn^3 females to wild-type males and the reciprocal cross were made. Spots due to somatic crossing-over were observed on more than 21 per cent of the F_1 females. Since c3G flies are indistinguishable from flies not containing this gene, contamination or errors during the building up or the keeping of c3G stocks do not automatically become obvious. Therefore, as a check on the purity of the c3G stock, crossover tests were made before and after the experiment. These tests confirmed the absence of crossing-over in all cases. The low viability of the stock and high percentage of nondisjunction in both chromosomes I and IV during the experiment also served as a check on the presence of c3G.

In spite of the presence of c3G which suppresses crossing-over in meiosis, not less than 22 per cent of the flies examined showed somatic spots due to mitotic crossing-over. This compares with a frequency of 21.5 per cent in the "control" (Table 1).[2] Therefore, the elimination of meiotic crossing-over is not associated with an effect on mitotic crossing-over in somatic cells of Drosophila females.

TABLE 1

	Flies examined	Flies with spots	Per cent flies with spots	Total no. of spots	Per cent spots	Description of spots		
						y sn	y	sn
Control	93	20	21.5	26	27.9	15	6	5
Experiment ...	71	16	22.5	18	25.3	13	4	1

The result reported is not entirely unexpected because (1) Friesen reported spermatogonial crossing-over in X-rayed males (thus, mitotic without meiotic crossing-over, since there is no crossing-over in the male); (2) Gowen (*4*), after X-raying c3G flies, found somatic spots (eye mosaics); (3) Stern (un-

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[2] Since these figures are much higher than those reported in other studies of the same somatic changes (*1, 7*), it might be mentioned here that though some of the spots involved as many as five hairs, many spots were determined on the basis of a single hair. Undoubtedly, however, differences between the genetic background of the flies used in different experiments also contribute to the variability of frequency of spots observed.

published) saw regular pairing in the oögonial cells of homozygous c3G flies; and finally, (4) Dr. Jack Schultz permits me to quote him as having observed regular pairing in the salivary glands and in the endomitotic nerve cells of the ovary of homozygous c3G animals.

By themselves, however, these former results are not decisive, since in (1) and (2) they could have been attributed to special X-ray effects and since regular pairing in (3) and (4) does not necessarily signify crossing-over. Only some data on autosomal somatic crossing in untreated males (7) are of similar significance as the data reported here, which give evidence of mitotic crossing-over in untreated females homozygous for c3G.

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Linkage and Crossing-over Between Black Pigmentation and Susceptibility to Induced Fibrosarcoma in Mice[1]

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Evidence has been published which indicates that germinal mutations have been induced in mice by chemical means. These germinal changes occurred in mice whose parents and grandparents had been injected subcutaneously with methylcholanthrene for a number of generations. More recently it has been determined that one of these germinal mutations (recessive brown pigmentation to dominant black pigmentation) has also apparently involved the genetic mechanism which determines, in part, susceptibility to fibrosarcoma induced at the site of methylcholanthrene injection. Thus, the black mutants possess a tremendously enhanced susceptibility to fibrosarcoma above the susceptibility possessed by mice of their ancestry or even their brown litter mates. Further unpublished evidence has shown that of the mice of the 15 inbred strains developed by the author and tested for susceptibility to fibrosarcoma, all, irrespective of genetic origin or relationship, that possess the black gene show a higher susceptibility to induced fibrosarcoma than any mouse of any of the strains possessing the brown gene. Thus, a linkage experiment is clearly indicated.

The F_1 from a cross between the original brown ancestral stock (NHO descent) and the black mutants possess the susceptibility of the black mutants (dominant inheritance). The F_1 generation consisted of 76 mice, all showing the dominant black. In the backcross generation to the recessive brown stock, 235 mice have been obtained and tested for susceptibility to induced fibrosarcoma. Of these, 121 were black and 114 brown (expected on Mendelian theory, a 1:1 ratio). The black backcross mice retain the susceptibility of the black mutants and the F_1's, whereas the brown backcross show the low susceptibility of the ancestral brown stock. Thus, linkage between black hair color and susceptibility to induced fibrosarcoma has been demonstrated.

The late survivors of the F_1 (that is, those mice living beyond 75 per cent of the total F_1's) show some degree of resistance, since it takes a longer time for them to develop fibrosarcoma at the site of injection. Therefore, if this tendency to greater resistance to fibrosarcoma is due to the loss of the hypothetical high susceptibility "gene" through the process of crossing-over, it ought to be possible to demonstrate this by the investigation of their descendants. This has been done, and when mice of one subline (of three separate ones tested) of the black mutant derivatives, suspected of possessing low susceptibility to induced fibrosarcoma even though they were black, were outcrossed to the ancestral brown stock, the F_1's (255 mice) showed the fibrosarcoma susceptibility of the resistant brown stock mice. A new black subline derived from these resistant F_1 black mice continued to show the low susceptibility of the brown mice.

The present evidence would indicate that on the "black" chromosome there is a gene that determines high susceptibility to induced fibrosarcoma; on the "brown" chromosome, a gene that determines low susceptibility or resistance to the same induced neoplasm. In the data reported here it appears that the process of crossing-over has occurred between the black gene and the S^{fs} (susceptibility to induced fibrosarcoma) gene. Thus, the evidence is accumulating that the intrinsic or genetic nature of resistance and susceptibility to induced fibrosarcomas is beginning to be indicated; that is, a mouse develops a fibrosarcoma following the subcutaneous injection of methylcholanthrene because it has a peculiar configuration in its genetic constitution determined by genes which apparently obey the same laws of Mendelian heredity (linkage and crossing-over) as the genes that determine hair pigmentation, etc. One of the genes, the S^{fs}, is on the same chromosome that carries the gene for black pigmentation.

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