THE RESISTANCE OF SCIARA (DIPTERA) TO THE MUTAGENIC EFFECTS OF IRRADIATION

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On the basis of the irradiation studies made on Sciara to date, a condition has been noted which is of general interest with reference to the mode of action of x-rays on the hereditary material, namely, an apparent resistance to the mutagenic effects of irradiation. Whereas gross and minute chromosome rearrangements are induced in treated germ cells, visible mutations appear at a negligible frequency.

Sciara is very unusual in this respect. In other organisms, including Drosophila, maize, and Neurospora, x-rays are found to induce both mutations and chromosome breaks at frequencies proportional to the dosage. During the past twenty years at least eight investigators have independently looked for visible mutations among the progeny of irradiated Sciara; altogether only twenty-four or twenty-six mutant characters have been obtained in the several species treated. Unfortunately, the irradiation data are not tabulated in such a way that an estimate can be made of the number of germ cells exposed or the total progeny examined. But certain of the studies were extensive. Both male and female germ cells were treated at various times in the developmental cycle and dosages from 3000 to 30,000 r. applied.

In view of the low mutation rate obtained repeatedly (see Metz, 1938), the chromosomes of Sciara were believed to be resistant to irradiation. The first clue to the contrary was the discovery of a reciprocal translocation in the salivary gland nuclei of larvae taken from cultures of the "Stop" mutant (Crouse and Smith-Stocking, 1938). The salivary gland chromosomes were then utilized in a cytological analysis of F_1 larvae derived from irradiated sperm or oocytes (Metz and Boche, 1939); following exposure of sperm to 5000 r., approximately 25 per cent of the F_1 showed gross chromosomal rearrangements. This unexpected induction of chromosome aberrations in Sciara was confirmed in subsequent experiments.

The experiments reported in this paper were performed in connection with cyto-genetic studies on the unusual behavior of the sex chromosome of Sciara. The data bear on the problem at hand, however, and will therefore be discussed in this relation.

Preliminary data on dominant lethal induction are in line with the rearrangement studies and provide further evidence that the chromosomes of Sciara are sensitive to irradiation. In one experiment designed to pick up X-translocations in *S. coprophila,* nine females were bred singly to adult males which had been x-rayed at 4000 r. The nine females yielded 108 total offspring, while nine control females from the same stock (isogenic) produced 723 total offspring. The exact probability (as measured by chi-square) of this difference is 0.0004. On the basis

of this small but very carefully conducted experiment, only 15 per cent emergence was obtained, a dominant lethal value which is practically identical to that measured by Demerec and Fano (1944) in Drosophila sperm x-rayed at 4000 r. It is of interest to note that in the Sciara experiment cited, nine of the 108 survivors (8 per cent) were heterozygous for X-translocations but none transmitted sex-linked visible mutations.

If dominant lethals are regarded as the result of certain types of chromosomal aberrations (see Pontecorvo, 1942), Sciara and Drosophila chromosomes respond to irradiation in a similar manner, and the physiological result (i.e., the lethal phenotype) is the same in both genera. It is with respect to less drastic physiological changes—namely, hereditary alterations classified as "visibles"—that the two genera differ.

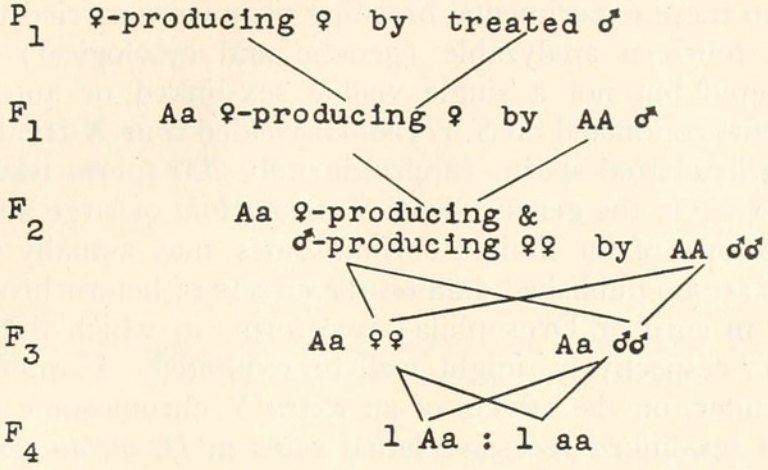
Several factors may account for the low visible mutation rate in Sciara.

(1) The external appearance of this fly (bristle pattern, pigmentation, etc.) is such that only the most conspicuous visible changes are likely to be detected.

(2) There is a distinct difference between the autosomal and the sex-linked mutations, which suggests that the induced mutation rate in this genus is considerably greater than the detected mutation rate. In *S. coprophila,* the species which has been most thoroughly worked, nine autosomal and five sex-linked mutations have been recovered. Of the autosomal group, seven are dominant and two are recessive; among the sex-linked factors, on the other hand, four are recessive and only one is dominant. The exact probability (as measured by chi-square) of this difference is 0.126. In material such as Drosophila, Habrobracon and maize, the dominant mutations constitute a very small percentage of the total number of visible mutations. The relatively high proportion of dominants in Sciara has been interpreted as evidence that this genus is unique in its response to irradiation (Metz, 1938). Such an interpretation is probably not valid, since, as noted above, the dominants constitute a majority only in the case of the autosomal factors. Most likely the discrepancy in the autosomal mutations has a relatively simple explanation, namely the extraordinarily tedious and inefficient technique available for the detection of autosomal recessives in this genus.

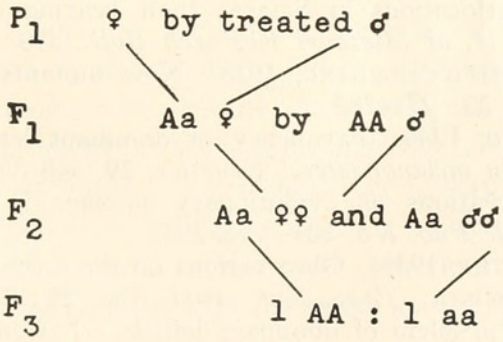
The mode of inheritance and the mechanism of sex determination in Sciara make it difficult to pick up autosomal recessive mutations. *S. coprophila* is monogenic. This means that the females produce either sons or daughters but not both; consequently, an F_1 female derived from an irradiated sperm will yield a family of sons or a family of daughters. If she is a male-producer and heterozygous for an induced sex-linked recessive, half of her sons should show the mutant character. Sex-linked recessives, therefore, can be fairly readily detected in Sciara in the F_2 generation. Autosomal recessives, on the other hand, are practically impossible to pick up because of the monogenic condition described above and because Sciara males transmit only the genes they inherit from their mother; the paternally derived chromosomes are eliminated at the first spermatocyte division. In searching for autosomal recessives, the best procedure is to take the mutated gene (a mutation induced in treated sperm), through the female germ line according to the scheme outlined below.

In order to detect the recessive mutation, a, four successive generations of flies (approximately four months at 70° F.) have to be produced as outlined; and then



the probability that the mutation will be detected in the F_4 is less than $1/16$. The calculation, of course, does not include I, the probability of induction of the mutation. In the sex-linked mutant strains of *S. coprophila,* male- and female-producers are phenotypically distinguishable. If one of these mutant strains is used in the experiment diagrammed above, the chance of selecting a ♀-producing female and a ♂-producing male of genotype Aa in the F_2 is $1/2 \times 1/2 = 1/4$ (F_2 females are AA or Aa). Then the chance of selecting a female and a male of genotype Aa in the F_3 is $1/2 \times 1/2 = 1/4$, making the final probability $I \times 1/4 \times 1/4$. Thus, the odds against detection of an autosomal recessive in *S. coprophila* are at least 15 : 1.

In digenic species like *S. reynoldsi* (the females produce both sons and daughters), autosomal recessives can be detected in the F_3 generation according to the following scheme:



In this case the chance of selecting an F_2 male and female of genotype Aa is $1/2 \times 1/2 = 1/4$, making the odds against detection of the mutation only 3 : 1 instead of 15 : 1.

The evidence presented so far is consistent with the view that the detected mutation rate in Sciara is considerably less than the induced rate. Even so, the induced rate appears to be less than that observed in Drosophila or Habrobracon; for, in the case of the sex chromosome of *S. coprophila,* where recessives can be picked up in the F_2, only five factors (four recessive and one dominant) have been found.

This dearth of mutants is particularly significant in view of the fact that over a period of several years the author has tested a large number of irradiated sperm (approximately 500) and oocytes (approximately 100) of *S. coprophila* in a search for reciprocal translocations which involve the X chromosome (Crouse, 1943 and

unpublished). In these experiments, breeding tests were carried to the F_2 generation or beyond; fourteen analyzable (genetic and cytological) X-translocations have been recovered but not a single visible sex-linked or autosomal mutation. Similar experiments conducted on *S. reynoldsi* yielded four X-translocations and no mutations among irradiated sperm (approximately 200 sperm tested).

(3) The presence in the germ cells of *S. coprophila* of large amounts of heterochromatin, in the form of the limited chromosomes, may actually retard the mutation rate. There are no published data on the effects of heterochromatin on general mutability either in corn or Drosophila—two forms in which the B-type chromosomes and the Y, respectively, might well be exploited. Unpublished studies of Miss Jean Kerschner, on the effects of an extra Y chromosome on the x-ray induced visible and sex-linked recessive lethal rates in *D. melanogaster* males, indicate that the extra Y brings about a significant decrease in these mutation rates.

(4) Finally, it is conceivable that, as compared to Drosophila or Habrobracon, the biochemical pathways which are available in Sciara result in a more restricted range of phenotypic variability.

Summary

Several facts have been discussed which may contribute to the apparent resistance of Sciara to the mutagenic effects of irradiation. New data are presented which (1) support the view that the visible mutation rate induced in this genus is lower than that found in Drosophila or Habrobracon; and (2) reveal a high dominant lethal mutation rate in this genus.

LITERATURE CITED

Crouse, H. V., 1943. Translocations in Sciara; their bearing on chromosome behavior and sex determination. *U. of Missouri Research Bull.*, **379**: 1–75.

Crouse, H. V., and H. Smith-Stocking, 1938. New mutants in Sciara and their genetic behavior. *Genetics,* **23**: 275–282.

Demerec, M., and U. Fano, 1944. Frequency of dominant lethals induced by radiation in sperms of *Drosophila melanogaster. Genetics,* **29**, 348–360.

Metz, C. W., 1938. Observations on evolutionary changes in the chromosomes of Sciara (Diptera). *Carnegie Pub. No.* **501**: 275–294.

Metz, C. W., and R. D. Boche, 1939. Observations on the mechanism of induced chromosome rearrangements in Sciara. *Proc. Nat. Acad. Sci.,* **25**: 280–284.

Pontecorvo, G., 1942. The problem of dominant lethals. J. Genet., **43**: 295–300.