OBSERVATIONS ON THE MECHANISM OF INDUCED CHROMOSOME REARRANGEMENTS IN SCIARA

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For several years efforts were made in this laboratory to induce mutations in the fungus fly, Sciara, by means of irradiation. These extensive studies, largely carried out by Dr. Helen Smith-Stocking,[1] seemed to show that the chromosomes of Sciara are peculiarly resistant to irradiation, as judged by the extremely small numbers of mutants secured and the high dosages tolerated. No adequate explanation could be given for such results, since both x-rays and radium were used and there was no reason to suppose that the rays failed to reach the chromosomes of the germ cells. In this work the flies irradiated were mostly females. Few males were treated, partly because of their poorer viability, but especially because in Sciara the chromosomes of males are not transmitted by their sons. The assumption was made from analogy with Drosophila,[2] that treating females would be as effective as treating males.

Since it is well known from the work of Muller,[3] Stadler[4] and subsequent investigators, that in general a correlation exists between induction of mutations and induction of gross chromosome rearrangements, it was postulated[5] that in Sciara rearrangements, like mutations, would show a low frequency. Such an expectation was heightened by the lack of gross chromosome rearrangements in hybrids between two species of Sciara.[6]

In order to get definite evidence regarding induced chromosome rearrangement we have recently carried out the experiments summarized below. Although these only represent the initial stages of the investigation they appear to warrant brief consideration at this time. Attention is confined here primarily to three topics: (1) The evidence, which indicates that chromosome rearrangements, and presumably mutations, may be secured readily by irradiating adult males (sperms), but not by irradiating adult females (unfertilized eggs). (2) The possible significance of the results in terms of the physical characteristics of the chromosomes. (3) The possible bearing of the phenomena on the question as to the relation between "gene mutation" and visible chromosome rearrangement. The latter two topics can only be treated very briefly, and with little discussion of other contributions to the subject.

We are greatly indebted to Dr. Louis R. Maxwell, of the Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, for the x-ray treatments, and to the staff of the Kelly Hospital, Baltimore, for the radium treatments considered here.

Chromosome Rearrangements.—In the studies on induction of visible rearrangements only x-rays were used. All treatments were made with the same apparatus and under as nearly uniform conditions as possible, using an air-cooled Coolidge tube with tungsten anode under a constant potential of 45 k. v., with no filter. The dosage is considered to be accurate within 5%. In one experiment the males and females were irradiated simultaneously.

In all cases the determinations were made through study of the salivary gland chromosomes of F_1 larvae, and all by one observer (Metz). Both male and female larvae were used. With the one exception noted below all cultures are from pair matings.

1. *Treated Males.*—Observations on treated males were extended only far enough to show the approximate frequency of rearrangements and the presence of rearrangements in numerous cultures. Four experiments involved a dosage of 5000 r units. From these 17 cultures were tested. All revealed rearrangements except four, from each of which less than seven specimens were examined. All are included in the following summary:

(*a*) *Sciara ocellaris* Comst. 18 larvae; 10 unaffected; 6 affected, 2 unfavorable for study; 7 rearrangements. (*b*) *Sciara coprophila* Lint. 48 larvae; 18 unaffected, 12 probably unaffected; 6 (+3 ?) affected, 10 unfavorable; 6 rearrangements (plus 3 doubtful cases ?). (*c*) Same species; mass culture. 25 larvae; 6 (+4 ?) unaffected; 2 affected; 13 unfavorable; 2 rearrangements. (*d*) *Sciara reynoldsi* Metz, 72 larvae; 42 (+5 ?) unaffected; 12 (+1 ?) affected; 12 unfavorable; 13 (+3 ?) rearrangements.

Total: Counting only the clearly unaffected and the clearly affected cases, there is a total of 28 rearrangements (14 translocations and 14 inversions) in 104 larvae, or approximately 27%. If doubtful cases are included the percentage ranges between 22 and 27 depending on the basis of classification. In addition, other males were given 3000 and 7000 r units, with correspondingly different results.

2. *Treated Virgin Females.*—Females were all given 5000 r units. Three experiments were carried out, and material was examined from 13 cultures. In order to avoid selection all, or practically all, larvae in the culture were used in some cases. *S. ocellaris:* 65 larvae; 52 unaffected; 13 unfavorable for study. *S. reynoldsi:* Lot 1; 96 larvae; 88 unaffected; 8 unfavorable. Lot 2; 55 larvae; 45 unaffected; 10 unfavorable.

Total: 185 unaffected; 31 unsatisfactory for study. Ten of those classed as unaffected were not entirely satisfactory for study but were apparently unaffected.

No evidence of rearrangement was found in any material from treated virgin females. The only suggestion of such an effect in our results is the finding of three rearrangements in a preliminary experiment on *S. coprophila* in which females not known to be virgin were treated.

These results seem contrary to those secured in Drosophila, where no such extreme difference has been found between the response of germ cells in the two sexes.[2] The difference, however, is probably not concerned with sex, but with other factors. The clue to the solution presumably lies in the fact that in Sciara, unlike Drosophila, the eggs of any female all mature at once and are all nearly mature when she emerges. Hence only mature, or nearly mature, eggs have been irradiated, whereas in Drosophila oöcytes and oögonia in various stages are all irradiated simultaneously.

Although the number of gross rearrangements (or chromosome breaks) recorded here from treated Sciara males is probably significantly lower than that secured in Drosophila,[7] it shows clearly that such rearrangements are readily secured. Their absence in material from treated virgin females, therefore, indicates either that rearrangements are not produced with anything like the same frequency in the eggs used, or else that in some way the altered chromosomes or eggs are eliminated. We have been unable to find any evidence that the latter alternative is correct. Fertility does not appear to be reduced more by treating females than by treating males. On the contrary, females will tolerate treatments much more severe than those which appear to completely sterilize the males. Apparently no weeding out of modified chromosomes occurs unless it is by selective segregation at meiosis in the treated egg. This feature is being investigated, but the possibility of accounting for the results on such a basis seems remote. The one translocation manifest by a mutant character ("Stop") in Sciara is readily transmitted through both eggs and sperms.

Assuming a Poisson distribution of breaks in eggs and sperms, calculations indicate that we should have recovered between 24 and 40 rearrangements. While these calculations involve numerous assumptions they possess the advantage of giving an estimate of the minimum frequency of rearrangements expected from x-rayed eggs. So far as we know this matter has not been studied in Drosophila, although the same reasoning is applicable.

It is believed that the present results from treated females reflect an actual absence or very low incidence of rearrangements. This agrees with the genetic evidence mentioned above, concerning induced mutation from treated virgin females. What is lacking at present is adequate genetic evidence from treated males (sperms). One experiment (Smith-Stocking, unpublished), however, suggests that mutation may readily be induced by treating sperms. Here a mixture of males and fertilized as well as unfertilized females (of *S. reynoldsi*) was treated with radium. Among 1073 offspring following a 5 gram-hour treatment 2 mutants were found. From a treatment of 3 gram-hours one was secured among 281 individuals. Several other possible mutants appeared, but they were sterile.

Physical Characteristics of the Chromosomes.—It is considered probable that the difference between the frequency of rearrangement in sperms and in eggs is due to a difference in the physical characteristics of the chromosomes. Little is known regarding chromosomes in the sperm except that they are packed relatively closely together, and are presumably not in a condensed condition comparable to that obtaining during mitosis. The close proximity of the threads should give excellent opportunity for broken ends, caused by irradiation, to unite in new combinations and produce rearrangements.

In the eggs, during the time under consideration, the non-homologous chromosomes seem clearly not to be in such close proximity as in the sperms. Some females were treated on the first, others on the second, day after emergence; all with the same result. When the eggs become fully mature, on the second or third day, the chromosomes are apparently always in metaphase or anaphase of the first meiotic division, and fully condensed. During the first day, however, they are difficult to stain, and we have not yet determined just what condition they are in. Preliminary observations suggest that each tetrad is in the form of a well defined, but somewhat diffuse, prochromosome-like body, the matrix of which is a relatively firm gel. If this is the case the gel may be sufficiently resistant to prevent broken ends of chromosomes from moving enough to form new combinations. Synaptic association, of course, may act similarly.

It seems not improbable, as suggested in an earlier paper,[8] that irradiation serves to lessen the viscosity of the matrix or sheath material, which normally insulates the chromosome threads, and that this factor, together with proximity and perhaps activity of movement, serves to determine the frequency with which rearrangements are induced. Such an effect may be localized at the regions of chromosome breakage and due to the same causes as the breaks. There is considerable evidence to indicate that both the amount and the viscosity of the matrix-sheath material differ noticeably in different cells and under different conditions. This might serve to account for such observed differences in susceptibility as those between dormant and germinating seeds, mentioned above, between germ cells in different stages of development,[9] and between young and old sperms,[10] as well as the well-known differences between cells in different stages of the cell cycle.[11]

The possible bearing of the present phenomena on the relation between chromosome rearrangement and "gene mutation" is under investigation. It need only be observed here that if both effects may be produced readily by treating sperms, but not by treating eggs, as the evidence suggests, the question is again raised as to whether there is any essential difference between the initial process in "gene mutation" and that which gives rise to chromosome rearrangement.

[1] Smith, H. B., *Proc. 6 Int. Cong. Genetics*, **2**, 187 (1932); Smith-Stocking, Helen, *Genetics*, **21**, 421 (1936); Metz, C. W., *Carnegie Inst. Wash. Pub. 501*, **275** (1938).

[2] See, e.g., Timofeef-Ressovsky, *Mutationsforschung in der Vererbungslehre*, pp. 60, 61, Steinkopf, 1937.

[3] Muller, H. J., *Proc. 6 Int. Cong. Genetics*, **1**, 213 (1932).

[4] Stadler L. J., *Ibid.*, **1**, 274 (1932).

[5] Metz, C. W. (*loc. cit.*), and Metz, C. W., *Genetics*, **24**, 105 (1939).

[6] Metz, C. W., and Lawrence, E. G., *Jour. Hered.*, **29**, 179 (1938).

[7] In terms of chromosome breaks we find approximately 50% in Sciara compared with 102 and 125% in Drosophila. Data for Drosophila from Catcheside, D. G., *Jour. Genetics*, **36**, 307 (1938) and Bauer, H., Demerec, M., and Kaufmann, B. P., *Genetics*, **23**, 610 (1938).

[8] Metz, C. W., these PROCEEDINGS, **20**, 159 (1934).

[9] Shapiro, N. J., *Biol. Zhur.*, **6**, 837 (1937).

[10] Offermann, C. A., *Genetics*, **24**, 81 (1939).

[11] See, e.g., Sax, K., *Genetics*, **23**, 494 (1938).

EVIDENCE FOR THE EXISTENCE OF AN ELECTRO-DYNAMIC FIELD IN LIVING ORGANISMS*

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There are two major classical theories of modern science: particle physics and field physics. The chief difference between them was clearly stated by Clerk Maxwell in his initial paper on electro-magnetic theory. Particle physics, he writes, considers any phenomenon "as due to the mutual action of particles," "but we are proceeding on a different principle, and searching for the explanation of the phenomena, not in the currents alone but also in the surrounding medium"[1] or, to use the language of his third paper, "in the form of the relations of the motion of the parts."[2] In short, particle physics directs attention to the constituent particles, whereas field physics centers theory and experimentation upon the medium in which the system as a whole is imbedded and upon its structure.

Since the fundamental problem of biology is organization, it would appear that field physics is the more appropriate for its investigation. It was considerations similar to these, together with certain facts in experimental embryology,[3] which caused the writers in 1935 to propose the "electro-dynamic theory of life."[4] It was this theory in turn which guided Burr, Lane and Nims to the construction of the vacuum-tube microvoltmeter,[5] and which suggested the experimental investigations [6–18] and findings which it is the purpose of the remainder of this paper to summarize.

In biology, the complexity of the living system is so great that investi-