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Induction of lethal mutations in *Drosophila melanogaster* by DNA

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Over two decades ago the writer (Gershenson, 1939, 1940) found that an addition to the food of *Drosophila* larvae of DNA isolated from calf thymus has a mutagenic effect. These experiments as well as later ones carried out jointly with other workers of the same laboratory (Gershenson *et al.*, 1948; Gershenson & Kisseliova, 1958; Kisseliova-Fedorenko & Miloserdova, 1963) showed that this treatment induces mutations mainly of several characteristic kinds. In contradistinction to other known mutagens it does not appreciably increase the frequency of sex-linked recessive lethals but leads to the appearance of a considerable number of visible mutations, both sex-linked and autosomal, most of which affect wing structure, some of the autosomal mutations in addition being of the Minute type. Recently Fahmy & Fahmy (1961, 1962) have obtained similar results by injecting DNA into adult *Drosophila* males.

A study of dominant autosomal mutations observed in our experiments showed that most of them were located in the 2nd chromosome and possessed a recessive lethal effect. Thus, though an addition of DNA to the food of larvae is virtually ineffective in producing sex-linked recessive lethals, it can induce such mutations in at least one of the autosomes.

The present paper deals with a further study of the induction by DNA of recessive lethals in the 2nd chromosome of *D. melanogaster*.

A highly inbred wild stock was freed from pre-existing recessive lethals in the 2nd chromosome. Larvae of this purified stock were reared on a potato–raisins–yeast medium with an addition of 12% (by weight) of a sodium salt of DNA isolated from calf thymus by Neumann's method. The method of isolation and characteristics of this DNA preparation were the same as described in our papers mentioned above. X-rayed male flies of the same purified stock (dosage 5000 *r*.) served as a control. Lethals in both the experimental and control series were detected by routine methods using dominant markers and *Cy* inversions which prevented crossing-over in the 2nd chromosomes.

450 X-rayed 2nd chromosomes and 750 derived from males reared on a medium with DNA were analysed. In order to avoid the influence of possible batches of mutations arising during spermatogenesis, only a single 2nd chromosome was

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investigated from each of the parents treated with DNA. Recessive lethals were found in 126 chromosomes from X-rayed fathers (28%) and in sixty-six of those from DNA-treated fathers (8·8%).

In each of the two series the lethals were tested for allelism. All 2145 possible combinations of crosses were performed with lethals found in the DNA-treated chromosomes. In the control series only 1400 combinations taken at random and comprising about one-fifth of all those possible were studied; these combinations included all the lethals found here. The results are presented in Table 1.

Table 1. *Results of intercrossing induced 2nd chromosome lethals*

Treatment	Number of combinations studied	Number of cases of allelism	Percentage of allelism
X-rays (control)	1400	55	$3{\cdot}9 \pm 0{\cdot}5$
DNA	2145	328	$15{\cdot}3 \pm 0{\cdot}7$
Difference			$11{\cdot}4 \pm 0{\cdot}9$

As seen from Table 1, most of the lethals in the X-rayed chromosomes were non-allelic whereas in the chromosomes of DNA-treated parents there was a high incidence of allelic lethals. A more detailed analysis of the data showed that practically all pairs of lethals found in the X-rayed chromosomes were different: among the lethals showing allelism each as a rule was allelic to only one other lethal (on the average to 1.1 lethals). The situation in the DNA-treated chromosomes is quite different: here whole groups of lethals were allelic one to another, i.e. each lethal was usually allelic to several or many (up to twenty-seven) other lethals (on the average to 9·6 lethals). Moreover, in many cases a lethal was allelic to two or several others which were non-allelic to each other, as seen in Fig. 1.

These results suggested the possibility that here some lethal mutations affect a larger segment of the chromosome while other allelic mutations affect smaller and sometimes overlapping parts of this segment, such cases resembling those of pseudoallelism.

To verify the above assumption it would be necessary to reassort the vertical columns of Fig. 1 in such a way that in all the horizontal rows cases of allelism would lie as close together as possible. This problem cannot be solved empirically because of the enormous number of possible sequences of the sixty-six vertical columns. However, as was shown by Dr V. V. Shkurba of the Institute of Cybernetics of the Academy of Sciences of the Ukrainian S.S.R. (1963), an algorithm can be found by the use of which an approximate solution of the problem can be achieved. On the basis of this approximation a further empirical analysis is feasible, giving several more or less equivalent arrangements of vertical columns with maximal clustering of cases of allelism in the horizontal rows. Figure 2 shows one of the best arrangements thus obtained.

An analysis of Fig. 2 leads to the conclusion that nearly all the lethals which arose in the 2nd chromosomes under the influence of DNA are located seemingly

in one continuous segment consisting probably of from thirty to thirty-five or thirty-six linearly arranged sub-units capable of mutating together or separately. Each lethal mutation usually affects from two to seven or eight neighbouring sub-units. Many of them show full complementation, as seen for example in Fig. 3 presenting data on allelism of twenty-eight lethals affecting seventeen overlapping sub-units.

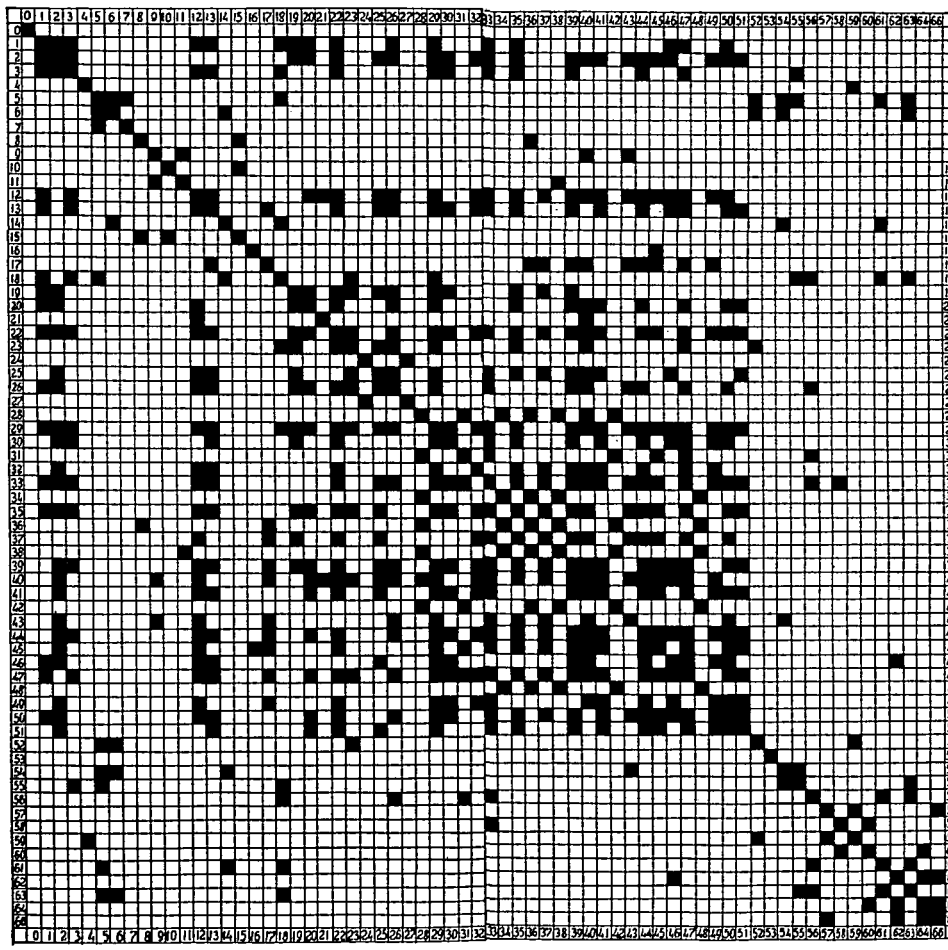

Fig. 1. Results of intercrossing all the lethals found in the DNA-treated 2nd chromosomes (black squares indicate allelism).

A complete map covering the whole affected segment of the 2nd chromosome and showing the exact sequence of all its sub-units could not be constructed because of contradictions caused by the fact that in a number of cases a lethal mutation affected evidently not one but two, or possibly even three, separate groups of sub-units, this leading to an uncertainty as to the position of about half of the sub-units. It is also possible that some of the crosses which gave an insufficient number of offspring were erroneously classified as showing allelism. The exact number of

flies from each cross was not counted but all vials containing visually less than fifty flies were discarded. However, some of those taken into account may have contained only *Cy* flies, not because of the lethality of the non-*Cy* homozygotes but as a result of random sampling, especially if one considers the somewhat lowered viability of these homozygotes carrying the 2nd chromosome from an inbred stock. Nevertheless, full complementation in such large groups of lethals as shown in

Fig. 2. Same data as in Fig. 1 after rearranging the vertical columns as explained in text.

Fig. 3 certainly speaks strongly in favour of the reality of linearly arranged sub-units in the affected chromosome segment. Only four of the sixty-six lethals (D-00, D-24, D-27, D-53) out of those which arose in the DNA-treated second chromosomes evidently do not belong to this segment as they are non-allelic to all others.

The distribution of lethals along this segment is non-random. Most of them affect several central sub-units and there exist at least two 'hot spots' which

contain most of the breaks dividing the mutated sub-units from the non-mutated ones.

Preliminary linkage experiments showed that the affected segment lies in the right arm of the 2nd chromosome in the vicinity of the Lobe locus. No cytological study of the chromosomes carrying these lethals has been undertaken, so no evaluation can be given of the size of this segment or of the sub-units which constitute it. However, this segment is probably rather small, as lethals representing an inactivation or deletion of considerable parts of it usually exert no influence on the phenotype of heterozygotes, and only a few of those affecting the largest number of sub-units show a slight Minute effect. It is not known whether the sub-units are separable by crossing-over.

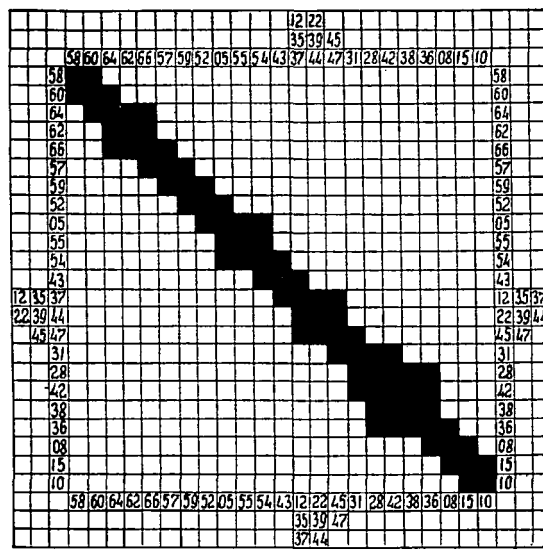

Fig. 3. Results of intercrossing twenty-eight lethals showing full complementation.

The above data show that an addition to the food of *Drosophila* larvae of a preparation of partly-depolymerized DNA from calf thymus induces recessive lethal mutations in the 2nd chromosome and, moreover, chiefly in only one limited segment of this chromosome, thus acting in a more or less directed fashion. As it is difficult to conceive any purely chemical mechanism which could lead to such a result, it seems likely that molecules of foreign DNA in some way interfere with the replication of definite regions of *Drosophila* chromosomes, in particular with the segment of the 2nd chromosome discussed here. However, some chemical alteration of the genetical material under the influence of the treatment applied seems also highly probable. Our previous work (1940, 1948) on visible mutations induced in *Drosophila* by the same treatment showed that it causes numerous mosaics and has a delayed mutagenic effect in subsequent generations. It is known that both these features are characteristic for chemical but not for physical mutagens (cf. Auerbach, 1946, 1950; Mathew, 1964).

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SUMMARY

Addition to the food of *Drosophila* larvae of DNA from calf thymus induces a considerable number of recessive lethal mutations in the 2nd chromosome. An analysis of sixty-six such lethals showed that most of them affect the same segment containing probably some thirty to thirty-five sub-units capable of mutating together or separately.

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