THE ORIGINATION OF CHROMATIN DEFICIENCIES AS MINUTE DELETIONS SUBJECT TO INSERTION ELSEWHERE

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I. EARLIER WORK ON THE NATURE OF INTERSTITIAL „DEFICIENCIES"

The phenomenon of so-called „deficiency", that is, genetic deficiency of a minute, interstitial (i.e., non-terminal) region of a chromosome map, was first met with by BRIDGES in the case of the forked-Bar region of the X-chromosome of *Drosophila*. It appeared as a spontaneous reversion of Bar to non-Bar accompanied by a lethal effect, and at the suggestion of the present author that the cause might lie in the loss of a piece of the chromosome, which would allow recessive mutant genes in the homologous chromosome to manifest themselves and would prevent crossing-over, the necessary tests were made by BRIDGES and showed that these effects were in fact produced. Unexpectedly, however, it appeared that the terminal (right-hand) region of the chromosome was present and normal, so that a simple breakage would not explain the results. As the phenomenon of deletion was not yet known, and seemed at the time a rather special assumption, it was deemed doubtful whether the region that appeared lost had really been physically lost, or had been inactivated or caused to undergo simultaneous mutation of all of its genes, chain-wise, to recessive mutant allelomorphs. At the same

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time (as was suggested by the present author) the results furnished evidence, from a new angle, of the physical validity of the linear arrangement of genes shown in the linkage maps, and indicated too that the normal allelomorph of Bar produced no other effect on the character concerned than did the mere absence of the mutant Bar gene.

The second case of deficiency, Notch 8, found and studied in detail by MOHR, was, in regard to the essential principles pertaining to it, similar to the first, although in another chromosome region and entailing a character effect (Notch wings) dominant to the normal. Here too it remained equally difficult to decide whether there was a real loss or inactivation or chain mutation, nor did the cytological methods of the time avail in reaching a decision. Similar considerations apply to the other natural interstitial deficiencies reported by BRIDGES since that time (Plexate, Minute 1, etc.).

With the coming of the X-ray technique and the direct proof through cytological evidence paralleling that of genetics, that deletions could occur which involved a breakage of the chromosome at two places, accompanied by reunion of the terminal pieces and loss of the middle piece from the combination, a mechanism was provided whereby the loss of non-terminal pieces could take place. This raised anew the question whether small deficiencies like the above, and others since found, had been caused in such a way. The cytologically demonstrated deletions, however, all involved the loss of the very large pieces; owing to the nature of the technique used, others would not then have been detectable. It was by no means a foregone conclusion that very small deletions also could take place. In fact, if an analogy were drawn from the process of double crossing over, such an extrapolation of the evidence from large deletions to small ones would not be justified, for in double crossing over it is well-known that two points that are far apart in the chromosome may readily have simultaneous breakage and reattachment, but not two points that are close together. It was therefore desirable to get further evidence concerning the nature of the small interstitial deficiencies, and this was forthcoming from several sources.

As one line of evidence, the author made an examination of several cases that had been obtained by PATTERSON, which seemed, from genetic evidence, to involve the loss, by simple breakage, of most or

all of the left-hand terminal region of the X-chromosome, extending to the right beyond the locus of Notch. (It may be remarked, however, that there was no real proof that the losses did extend entirely as far as the left end and so the cause might not have been a simple breakage). The data showed that these supposed losses had genetic effects like those of MOHR's Notch 8 deficiency (and, of course, the additional lethal effect due to the absence of PATTERSON's „viability gene" or genes, which had to be compensated by the addition of a partly complementary fragment, called „Theta", from the extreme left end). It was hoped that cytological proof could be obtained that in these cases, giving phaenotypic effects like those of previous „deficiencies", there was a real absence of a section of chromatin, but unfortunately the cytological attack failed, because the pieces involved were too small to be demonstrable by the methods then available. It must of course be conceded that, even if the real absence of a terminal piece had been cytologically demonstrable in these cases, this line of evidence for the loss interpretation of interstitial deficiencies must have remained an indirect one, since it could only show that the original deficiencies of MOHR, etc., gave genetic effects phaenotypically like those of these losses. Similar indirect proof from a converse direction was afforded by the author's studies of the effects of cytologically demonstrable extra pieces of the X chromosome, in which it was found that the phaenotypic results were opposite in character to those produced by the original „deficiencies", thus indicating that the latter comprised absences of the corresponding regions.

Another line of evidence lay in the analysis of cases of inversions and translocations, in which it was found that two breaks in the same chromosome might occur that were at distances from one another considerably smaller than was usual in the case of double crossing over, although cases were not yet known in which the distance was so small as to be comparable in size with those of previously studied „deficiencies".

While the above investigations were in progress, cytological work of MCCLINTOCK on maize did reveal cases in which there was actual absence from a chromosome of a comparatively small interstitial region. It would, to be sure, have been difficult to determine the lengths of these deficiencies in genetic units and to compare them

quantitatively with the lengths of the *Drosophila* deficiencies, but the general principle, so far as it applied to maize at any rate, was directly established.

The same principle has now been established in *Drosophila* by genetic methods in the case of scute 19, here to be described. In addition, after these findings on scute 19 had been made, and the present paper nearly completed, the new cytological method of PAINTER became available, whereby through a study of the uncoiled and reduplicated chromonemata in the salivary glands (as KOLTZOFF has shown them to be), differences of the degree of fineness of many interstitial deficiencies would be directly visible. By this means, as MACKENSEN has reported in a recent abstract, the fact that these deficiencies involve real absences has been clearly demonstrated. The same has likewise been demonstrated in later cytological work, following the method of PAINTER, that has been done by PROKOFYEVA at the Institute of Genetics (MULLER and PROKOFYEVA, in press), as well as in such work by KOSSIKOV and MULLER (in press). At the same time it still seems worth while to publish the account of the genetic investigation of scute 19, not only because this had led to the independent establishment of the same conclusion from the genetic side, but also because a number of significant facts are brought out in this case that are not to be gained simply by the cytological analysis of cases of deficiency.

II. THE SCUTE 19 TRANSLOCATION — FIRST RESULTS

In a number of laboratories translocations have been extensively studied that involve the mutual interchange of terminal pieces between non-homologous chromosomes, and, where these pieces are small, the aneuploid offspring resulting from recombination taking place in parents heterozygous for these translocations are frequently found to be viable and to show phaenotypic effects similar to those in individuals having „deficiencies" of the same region as that which the aneuploids are known to lack. Hitherto, however, such studies have not been reported in cases of translocations which seemed the same in method of formation as natural deficiencies of the type at issue, that is, in cases involving minute non-terminal (= „interstitial") regions.

The first case in point in which it could be positively proved by genetic evidence that a minute non-terminal region had been deleted from a chromosome is that involving the „mutation" scute 19. It can be definitely proved in this case by genetic evidence that the region in question has not merely mutated chainwise or been inactivated, but that it has actually been lost from the chromosome from which it lay, since the presence of the genes in question can now be demonstrated in a non-homologous chromosome, into which they have become inserted.

Scute 19 was found by LEAGUE as a scute mutation in some radiation experiments which she was carrying on under the direction of the author. The chromosome containing the „delta 49" inversion, and bobbed but otherwise normal genes, had been irradiated and crossed to yellow females with attached X's, and among the male offspring there was one which had the scutellar bristles absent and the sternital bristles reduced in number, as in the case of the mutation scute 5. Stock derived from it bred true to these characters, and the further analysis which we then made showed: 1) that the X-chromosome was broken a little to the right of scute; 2) that the piece that had been at, or near, the left end, containing the locus of scute 19 and the normal allelomorph of yellow, was attached to the second chromosome in its left-hand region; 3) that heterozygous aneuploid females of both classes could live, i.e., those deficient for one dose of the small piece and those having an extra dose of it; deficient males died, but those having an extra dose of the piece could live.

In order to determine whether there had been a mutual exchange of materials between the X-chromosome and the second, a test was made by the author of whether females could live, both of whose X-chromosomes were normal in the respect in question (attached X-chromosomes were used) and both of whose second chromosomes had the scute 19 pieces attached to them. It was found that they could live. The test was made by crossing yellow attached-X Curly females to scute 19 males, and then crossing together the resulting scute 19 Curly males and the non-yellow, Curly females. Among the offspring, non-yellow, non-Curly females occurred, and these must have been homozygous for the second chromosome carrying the loci of non-yellow and of scute 19, although they were also homozygous

for the attached X's. Likewise it was found that males could live which had a normal X, but two second chromosomes containing the scute 19 piece. If the second chromosome having this attached piece of the X, had in exchange given up some of its material to the X from which the above piece had been taken, then the above individuals would have completely lacked the portion of the second chromosome thus removed, since both their second chromosomes were of the type in question, and their X-chromosomes, being of another origin, could not have contained the region of the second chromosome that had been translocated. Such individuals then, homozygous for the absence of a part of an active region, would be expected to die, or at least to show some abnormality, instead of being viable, fertile and phaenotypically normal, as they were. The conclusion was accordingly drawn that the exchange had not been a mutual one, the second chromosome having been recipient only, and the X donor only. The case, then, so far resembled one of simple translocation.

Further tests showed that the small translocated piece of the X-chromosome, when present as an extra piece, failed to „cover" any of the known loci to the right of scute — that is, it did not dominate over the recessive genes in these loci. Among these loci is, for example, the ommatidial disarrangement (*om*) which had been found by the author in stock of scute 10 (achaete 3), and is closely linked to the scute locus. All scute, achaete and yellow recessive allelomorphs were dominated over by the piece, with the exception, of course, of bristle absences identical with those in scute 19 itself, though even the latter were made somewhat more normal by the extra dose. The lethal called 1J1, which arose simultaneously with scute J1, and is very closely linked to it, was not dominated over. In the case of all these tests, a double check was carried out, since individuals of two complementary types were obtained, both of which carried one X-chromosome with the recessive mutant gene in question, but had in addition, in the one case, the extra fragment bearing scute 19 itself (these were, of course, males), and, in the other case, the remainder of the X-chromosome from which the fragment had been removed, and not the fragment itself (these being females). Of course, it was always found that if the fragment dominated over the gene in question, the rest of the chromosome did not, and vice versa, *i.e.*,

the effects of the two parts of the broken X were quite complementary.

III. PROOF OF THE NON-TERMINAL CHARACTER OF THE SCUTE 19 TRANSLOCATION

Although the possibility was recognized from the first that the small fragment might be non-terminal (see MULLER, 1932), this possibility was hardly considered probable, in view of the nearness of yellow and scute to the end of the chromosome, and it was accordingly thought that lethal Jl lay to the right of scute, beyond the break of the scute 19 chromosome. Further work has shown, however, that lethal Jl lies to the left of scute and of yellow, and that therefore the fragment cannot extend as far as the left end of the X-chromosome, since it does not cover this locus.

The determination of the position of lJ 1 with respect to that of scute was made by breaking the scute 8 inverted X-chromosome by means of X-rays in its left hand inert region, thus obtaining fragments which, so far as the active region was concerned, contained on the one hand only the extreme left end with the loci of yellow and achaete and those to the left of them, and on the other hand the remainder of the chromosome, with these loci absent. Various such cases were obtained. In one of them there was a mutual translocation to the fourth chromosome, so that it was certain that the two pieces of the X were quite complementary. In other cases the large right-hand remainder of the X was obtained by apparently simple fragmentation, involving the loss of the left end. Deleted X-chromosomes containing the left end, and little or none of the right end, were also obtained. As was to be expected on the basis of previous investigations of SEREBROVSKY into the nature of the scute 8 inversion, confirmed by investigations of LEVIT, of GERSHENSON and of the present author, the left hand pieces of the scute 8 chromosome dominate over yellow and achaete recessives, but not over scute proper, while the right hand portions, conversely, dominate over scute, but not over yellow and achaete. In crosses to stock having scute J1 and its associated lethal J1, it was found that the left fragments dominated over the lethal and allowed males with this lethal and the scute J1 gene to live, and to manifest the scute J1

character, while, conversely, females containing the chromosome with lJ1 and scute J1, and containing in addition a broken scute 8 chromosome lacking the left end, failed to appear. This then placed the locus of lethal J1 to the left to scute.

The locus of lethal J1 was then proved to be even to the left of yellow, in the following way. A chromosome containing the yellow 3P inversion, which tests of the author had shown to have the locus of yellow in the inverted section, and therefore to the right, at the spindle fibre end, was allowed to cross over with a scute 8 X-chromosome, from which the left end had been removed by breakage. In this way a chromosome was obtained like the yellow 3P chromosome, but lacking the left end. It contained the locus of yellow and all loci which normally lie to the right of the latter, but no loci that are normally to the left of yellow (for further evidence on this point, see parallel paper on viable deficiencies). Such a chromosome in combination with a lethal J1-containing chromosome formed an inviable zygote. Thus the lethal J1 was to the left of yellow. As the evidence given in the parallel paper shows, the left-hand break of the X-chromosome in the case of scute 19 must lie immediately to the left of yellow. In the region to the left of this break must lie not only lethal J1 but also the locus of chlorotic (sometimes called „yellowish"), discovered first in 1919 by MOHR (1923) and again in 1923 by MORGAN (1929), which has one tenth percent crossing over with yellow. Its locus is almost certainly different from that of lethal J1, because although it itself is more or less semi-lethal, its phaenotypic effect seems quite unrelated to that of lethal J1. It causes a light body colour, but can be bred, whereas lethal J1, in the one viable male yet seen containing its locus, not „covered" by an extra fragment, manifested itself only in a disarrangement of the ommitidia and sterility, but had high viability of the adult. Unfortunately, there is no stock of chlorotic extant, allowing of further tests of its locus.

Further evidence of the fact that the translocated fragment containing scute 19 did not include genes to the left of yellow, is afforded by crosses in which individuals were made up that did not contain the fragment but contained one X-chromosome (designated as sc^{19-}) from which the fragment had been removed, and another X-chromosome of the type above described, namely, having the

yellow 3P inversion but having the left end of the chromosome, including not only the locus of lethal J1 but all loci from the left end up to (but stopping short of) yellow, missing. Such females were quite viable and fertile and showed only such slight phaenotypic deviations as are typical of flies having but one dose of the achaete and scute loci. Of course this interpretation depends upon a correct knowledge of the locus of the yellow 3P break. This is being discussed in another paper, where the characteristics of those females also are reported which contain on the one hand the deficient scute 19 chromosome, and on the other hand a scute 8 chromosome lacking the left end. The facts in question show the correctness of the present interpretation and thus prove again that the deficient scute 19 chromosome has the entire left terminal region present up to, but not including, the locus of yellow.

IV. THE INSERTION OF THE DELETED SECTION INTO CHROMOSOME II

It having thus been shown that the scute 19-containing fragment was a non-terminal piece, involving a break both left and right of it, it became probable that this piece had not undergone either a simple terminal or a side attachment to the second chromosome, but had become attached to the latter by both broken ends („surfaces of fracture"), a process which would require a breakage on the part of the second chromosome also, for the reception of the piece, *i.e.*, „insertion". In that case the locus of scute 19 would behave as though lying at some non-terminal position on the linkage map of chromosome II. Tests were therefore carried out to determine this linkage. Attached-X females were produced having one second chromosome containing the „all" series of recessive markers (which include, among other genes, aristaless, dumpy and black) and the other second chromosome containing the scute 19 fragment. These were crossed to males heterozygous for the „all" series and for Curly. Among the female offspring of this cross, non-yellow (versus yellow) served as a marker for the scute 19 fragment, and among the non-Curly offspring the distribution of the other markers also could be seen. The results showed that the fragment was indeed attached non-terminally, one or two units to the right of dumpy, that is, at about locus 13. The crossing over between the markers was not

perceptibly reduced, nor in any wise abnormal, the piece evidently being too small to affect crossing over appreciably. While it cannot be categorically proved that the case is not one of side attachment, this becomes very unlikely in view of the apparent great rarity of the latter phenomenon, if it exists at all [1]), combined with the fact that the attached piece had been broken at both its ends. Insertion is the only probable explanation here.

V. THE GENETIC SIZE OF THE DELETED AND INSERTED SECTION

It becomes of interest at the same time to examine more exactly the genetic extent of the fragment that had been thus deleted and inserted. Since, as shown in a parallel paper, achaete and scute are now known to be separate, there must be at least three genes in the fragment, namely yellow, achaete and scute. Yellow and achaete have not, as a matter of fact, yet been separated by crossing over or breakage, but their dependence upon separate genes is extremely probable in view both of their very different phaenotypic effects and of their usually separate mutations.

Further examination of what loci may be present in the piece was aided by an intensive study which the author has been carrying on into the limits of divisibility of the region of the chromosome in question. This entails a comparison of the position of the breaks in various translocations, inversions, etc., of the region. Without going into the details of this matter here, it must be stated that the work thus far has demonstrated at least one more locus, lying to the right of scute, in the region included by the scute 19 fragment, a locus whose absence is lethal in its effect. It is quite possible, and indeed probable, that there are more of these loci, which further study may demonstrate. The existence of the locus just mentioned is demonstrable if crosses are made between flies with the scute 4 and scute 9 inverted chromosomes. A crossover chromosome thereby obtained, which has the left portion of the scute 4 chromosome and the right portion of the scute 9 chromosome, is lethal in its effect upon a male, unless the scute 19 fragment, attached to chromosome II, is present

[1]) Since the above was written, evidence has been obtained by KOSSIKOV and MULLER (paper in press) that even the supposed classical case of side attachment — „Pale" translocation — in fact involves insertion instead.

in addition; in the latter case, the male lives. The lefthand breaks of scute 4 and scute 9 are both to the right of scute, but that of scute 9 must be some distance further to the right than that of scute 4, so that in the crossover chromosome the region between these two breaks is missing; but this missing region is supplied when the scute 19 fragment is added. This region, then, must contain at least one gene necessary for life.

Similar studies are being carried out in the attempt to divide the region still further if possible. In this way it is hoped eventually to be able to form some estimate of the actual number of genes in the scute 19-containing fragment. The hope of being able to make such a determination is raised by the fact that the left-hand limit has already been precisely determined. Whatever the answer may be, however, the fragment should physically be a very small one, since it could not be shown to cover any other visible genes than those mentioned, in spite of the fact that all deleted chromosomes previously tested, that extended to the right of scute, covered at least the locus *om*, even when they did not cover PATTERSON's „viability gene".

VI. DISCUSSION

The reasons have been presented in another paper (MULLER, 1932) for considering the two breaks in cases of double breaks as not independent of one another, but as both dependent upon some process that accompanies chromosome contact under special conditions, such as X-rays may bring about. The process may in some ways be compared to crossing over, involving as it does reattachement at the point of contact, but being a kind of illegitimate crossing over between non-homologous regions that ordinarily do not touch in this way. A further difference from crossing over is now demonstrated clearly in the fact that two points of breakage in the same chromosome may under these conditions be so near together, thus being free from the operation of the principle of interference demonstrated by the author in 1912 to hold in the case of crossing over. Interference, so far as present knowledge goes, is apparently explained most plausibly by considerations of chromosome rigidity obtaining under ordinary conditions. Why such interference should not operate

in the case of these breaks and reattachments between non-homologous chromosomes is a new problem. As mentioned in a previous section, there were already indications that the rule did not operate normally in such cases, based on the comparatively short distance between the two breaks in some already known cases, but in no case known was the distance of the order of minuteness of that in scute 19. It would be of interest to know if such cases may be actually of higher frequency of occurrence (in relation to the number calculated on the basis of no interference) than those in which the breaks are somewhat further apart [1]).

Another respect in which the present case shows the operation of a principle different from that found in crossing over, lies in the fact that there was here no real exchange of parts, inasmuch as one chromosome was only donor and the other only recipient. The first chromosome broke at two points, the second at not more than one point. This could not have come about through a mere exchange of attachments, at points of breakage coinciding with points of contact, unless the first chromosome had been looped upon itself in a most minute loop, and the second chromosome had rested against the first at *precisely* the point of crossing of this loop. OFFERMANN has suggested that this apparent coincidence might have occurred through the second chromosome having slid along until it naturally came to rest in the notch of the first. At any rate, it is not a case of the simple crossing at two points, of two threads, A and B, followed by their breakage and reunion, for in that event if one part of A becomes attached to one part of B, the other part of A must become attached to the other part of B, as happens in crossing over. The latter test of the translocation, inversion and deletion hypothesis of SEREBROVSKY and DUBININ, suggested in a recent paper of the author (1932), therefore receives a negative answer. Nevertheless, because of the possibility of contact of three strands at one point, above discussed, this can no longer be regarded as invalidating the very probable idea that chromatin contacts are a causative factor in the recombination process.

The question now arises, are all deficiencies of non-terminal regions produced in essentially the same way as scute 19, by the

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[1]) Evidence to this effect has since been obtained.

deleted region having become entangled with another chromosome region? Certainly in some cases at least deletions could arise this way, in which the piece deleted out was never found, for the latter would have a chance to become lost at the subsequent mitotic division, if the process had occurred at a stage when the chromosomes were split, and it would at any rate have another chance at a subsequent meiotic division. On the other hand the piece, although deleted out by contact with another region, might conceivably fail to become attached to any portion of the latter. Again, the mere looping of a single chromosome upon itself, after the manner of an inversion, might have sufficed to entangle its parts sufficiently to cause the deletion, and the part deleted out might, at least at times, have its ends join one another to make a ring; this would lack a fibre attachment point and would therefore be incapable of mitotic transportation. The loopings here in question probably correspond to the very fine spirals described by VEJDOVSKY in 1911.

If these cases can occur, then it is also possible that equally minute inversions may occur, which could scarcely be recognised by the test of reduced crossing over, but which might nevertheless involve apparent mutations, dependent upon the „position effect" of the rearranged genes. This involves us in the question of how many supposed gene mutations may really be of this type. We are now studying this matter by means of tests of a special kind. The answer to it is critically bound up with the whole problem of gene mutation [1]).

A further point of interest brought out in the scute 19 case is the viability of the individuals homozygous for the small extra fragment attached to the second chromosome, when they already have this region represented in normal X-chromosomes. Such individuals live comparatively well and can be bred, and no doubt could be brought practically to normal viability by selection of „modifying" genes. They show a somewhat darker colour and tendency towards extra bristles, due to the extra doses of the loci concerned (of yellow, achaete and scute), but the piece is so small that it is easily conceivable that such duplications could become established in natural evolution. This then is one method by which the number of genes could become increased. Aneuploidy caused by the addition of

[1]) Since the above has been written, proof of the occurrence of such inversions has been obtained by MULLER, PROKOFYEVA and RAFFEL (in press).

whole chromosomes is usually too detrimental to play such a role (except where polyploidy has preceded it). Following such duplication, it is to be expected that the redundant loci will come to have divergent mutations established in them in the course of evolution, and so gradually will become more differentiated, until they can finally be regarded as quite non-homologous genes. In the meantime, however, they would show some ability to produce parallel or „duplicate" mutations and some synaptic affinity.

While such duplications of regions might sometimes become placed in non-homologous chromosomes, as in the scute 19 case, it is to be noted that the likeliest point of attachment, ordinarily, would be in a nearly homologous position in a sister or homologous chromosome, since these ordinarily lie so close together in the cell. Here, then, a localized duplication of materials could be produced, giving rise to an effect similar to that produced by unequal crossing over. Of course this conception is rather speculative, but the possibility of it is of interest in connection with other questions raised by the study of the achaete-scute complex.

Attention may hereby be called to the paper by MULLER and PROKOFYEVA, in which cytological examination of the scute 19 deficiency is made, with the object of determining the method of cytological expression of the genes in the chromosomes of resting nuclei (salivary glands). It may be stated here that the results fully confirm the genetic finding of the deletion of an extremely minute interstitial region of the X-chromosome, located near its left end.

SUMMARY

1) The scute 19 translocation involved the breakage of the X-chromosome, under the influence of X-rays, at two points very near together. Between these points there were located the loci of yellow, achaete, scute and at least one other gene, whose absence acts as a recessive lethal.

2) The minute region between these two breaks was deleted from the X-chromosome, the remainder of which joined together again.

3) The region thus deleted became attached to a non-terminal region of the second chromosome, close to the right of the locus of *dp*, probably by breakage of the second chromosome and insertion of the

deleted piece between the resulting pieces of the second chromosome, accompanied by chainwise union of the three pieces.

4) The translocation was not mutual, for no genes became transferred from the second chromosome to the X. The explanation of this fact seems to require the meeting of three threads at one point, presumably by one becoming caught in the notch made by the crossing of the other two.

5) The X-chromosome with the small missing section, when in a combination containing normal second chromosomes without the inserted fragment, gives the results typical of a so-called „deficiency".

6) On the other hand, individuals having the second chromosome with the attached fragment, but normal X-chromosomes, exhibit the phenomena typical of so-called „duplications", although, unlike what is found in most cases of duplications involving larger regions, individuals homozygous for these duplications can live and breed.

7) One method of origination of minute interstitial „deficiencies" is thus illustrated, and it is proved that these deficiencies involve real absence. Problems connected with the method of formation of such minute deletions are discussed.

8) The question is raised of to what extent supposed gene mutations may consist of similarly minute inversions, arising by a similar process.

9) It is pointed out that the ability of such minute portions of chromatin to become transferred to other regions makes possible also the origination of reduplications of gene material, that are viable and that might become established in evolution.

The results of cytological examination of the scute 19 case are presented in a parallel paper by MULLER and PROKOFYEVA.

LITERATURE CITED

BRIDGES, C. B., 1917. „Deficiency". Genetics 2: 445—465.

KOSSIKOV, R. V. and H. J. MULLER, 1935. „Invalidation of the genetic evidence for branched chromonemas in the case of Pale translocation". J. Hered. (in press).

MACKENSEN, O., 1933. „A cytological study of short deficiencies in the X chromosome of *Drosophila melanogaster*". Rec. Gen. Soc. Amer. 2: 16.

MCCLINTOCK, B., 1931. „Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*". Mo. Agric. Exp. Sta. Bull. 163: 30 pp.

MOHR, O. L., 1919. „Character changes caused by mutation of an entire region of a chromosome in *Drosophila*". Genetics 4: 275—282.

MOHR, O. L., 1923*a*. „Das Deficiency-Phänomen bei *Drosophila melanogaster*". Z. f. ind. Abst. u. Vererb. 30: 279—283.

MOHR, O. L., 1923*b*. „Modifications of the sex-ratio through a sex-linked semi-lethal in *Drosophila melanogaster*. (Besides notes on an autosomal section deficiency)". Studia Mendeliana, Brunn: 1—22.

MOHR, O. L., 1923*c*. „A genetic and cytological analysis of a section deficiency involving four units of the X-chromosome in *Drosophila melanogaster*". Z. f. ind. Abst. u. Vererb. 32: 108—232.

MOHR, O. L., 1927. „Exaggeration and inhibition phenomena". (Abstr.) Verh. d. V. intern. Kong. f. Vererb., Sup. Bd. II d. Z. f. ind. Abst. u. Vererb., publ. 1928: 1135.

MOHR, O. L., 1929. „Exaggeration and inhibition phenomena encountered in the analysis of an autosomal dominant". Z. f. ind. Abst. u. Vererb. 50: 113—200.

MOHR, O. L., 1932. „On the potency of mutant genes and wild-type allelomorphs". Proc. 6th Intern. Cong. Gen. 1: 190—212.

MORGAN, T. H., 1929. „Data relating to six mutants of *Drosophila*". Carn. Inst. Wash. Publ. 399: 169—199.

MORGAN, T. H., C. B. BRIDGES and A. H. STURTEVANT, 1925. „The genetics of *Drosophila*". Bibliographia Genetica 2: 262 pp.

MULLER, H. J., 1932. „Further studies on the nature and causes of gene mutations". Proc. 6th Intern. Cong. Gen. 1: 213—255.

MULLER, H. J. and A. A. PROKOFYEVA, 1934. „Continuity and discontinuity of the hereditary material". C. R. d. Acad. Sc. d. U.S.S.R. 4 : 74—83.

MULLER, H. J., A. A. PROKOFYEVA and D. RAFFEL, 1934. „Minute intergenic rearrangement as a cause of apparent gene mutation." Nature (In press).

PATTERSON, J. T., 1932. „A gene for viability in the X-chromosome of *Drosophila*". Z. f. ind. Abst. u. Vererb. 60: 125—136.

SEREBROVSKY, A. S. and M. G. ZUBINA, 1930. „About the limits of dividing capacity of the scute gene". Trans. of Fourth Cong. of Z., A. and H. of U.S.S.R.

VEJDOVSKY, F., 1911. „Die Vererbungsträger". Prag.