THE INDIVIDUAL GENE IN RELATION TO THE CHROMOMERE AND THE CHROMOSOME*

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We have endeavored during the past year to determine, by analysis of a selected lot of chromosome breaks produced by irradiation in Drosophila, to what extent the chromosome is subdivisible into its constituent genes (or perhaps still further?), the cytological basis of the constituents thus separated out, and the functional consequences of such separation. Results have already been attained which throw some light upon all of these problems. They are being described in detail in a series of papers in press, and the present article presents only a preliminary survey of them.

In the present work, attention has been mainly concentrated upon a highly restricted region of the chromatin, in which as many cases of breakage as possible were analyzed with reference to one another, in order to determine what limitations there might be in regard to the positions and manner of breakage. The basis for selection, i.e., the criterion that the break lay within the narrow limits desired, consisted in the phaenotypic effect, accompanying breakage, which was produced on one of the characters ("scute," "achaete" or "yellow"), affected by the region in question. The essence of the method of analysis (Muller1) consisted in making recombinations between the various cases of breakage, taken two at a time, with the resultant production of individuals containing the portion of the chromosome to the left of one of the two breaks, together with that to the right of the other break. If the first break was to the left of the second one, this recombinational individual would necessarily contain a deficiency for the region lying between the two breaks, and would hence tend to die or be abnormal, while the complementary recombinational individual (containing the portion of the chromosome to the left of the second break, and that to the right of the first break) would carry a duplication of the genetic material lying between the two points of breakage, a circumstance not nearly so upsetting to the soma. Mutatis mutandis, if
the second break were to the left of the first, the reverse phaenotypic relations would be observed. If the two breaks were in identical positions or in positions so nearly identical that a deficiency of the region between them was without detectable effect on the soma, then neither of the complementary classes of recombinations would be lethal or give the appearance of abnormality. In the actual working out of this method many complications arose, the details of which cannot be mentioned here; these depended on the fact that the breakages involved reattachment of the broken fragments at another point in the chromatin than before and this was nearly always at another point of breakage; thus deficiencies and duplications arose in the region of this other breakage as well, and had to be overcome in some way or else allowed for.

Thus far seven breaks in the chosen region have been analyzed with reference to each other (and an eighth break has been analyzed with reference to six of the former seven). Among these seven cases, only four surely separate positions of breakage have been found. In other words, three of the breakages were either in exactly the same position as previously studied breakages, or in so nearly the same position that a complete deficiency of the region between the two points of breakage was practically without phaenotypic effect. Nevertheless, the other breakages were far enough apart, so that a deficiency of the region between any two of them was productive of a drastic phaenotypic effect (lethal in all cases but one). The conclusion therefore becomes probable that there are only certain fairly definite positions at which the chromatin may ordinarily be broken by irradiation, and that, by the methods here used, it is possible to discover the totality of these positions within a region circumscribed in the way explained. The blocks of material between these positions may be regarded as "genes" and, if this is true, the distances apart of the dissimilar breaks studied in an experiment of this kind are of the same order of magnitude as the individual genes, and thus the absolute number of genes contained within the restricted region is determinable by prolonging the experiment until it has become probable, by reason of the number of recurrences, that each possible position of breakage has been represented by at least one breakage. The present experiments are being continued with this objective, but in the meantime it is evident that the number of genes in the region between the two most distant breaks found in the experiment is at least of the same order of magnitude as the number of blocks thus far found, and probably not much more than twice that number.

At the same time evidence has not yet appeared that a gene—even the mooted gene for "scute"—can be divided within the limits of its own structure, in such a way as to permit the continued life of both separated portions. Further work of this kind should decide this question definitely.
The results, on the other hand, do indicate that even at the present level of fineness of resolution, the genes are arranged in line, i.e., in single file, inasmuch as all the recombinations gave results consistent with one another in showing the same gene order.

A further line of evidence for the conclusion that the distances between the breaks in question were of the order of magnitude of individual genes emerged from the finding that one of the deficiencies of a region between two near-by breaks was not lethal in its phaenotypic effect, but produced conspicuous visible abnormalities, corresponding to previous inferences regarding the visible effects which would be produced by absence of just two specific genes (those for "yellow" and "achaete") lying in the region in question. Here then there is little doubt that the two breaks considered were separated by the space of just two genes.

The total cytological extent of the entire region studied in this experiment was determined by Prokofyeva through observations of the chromosomes in the salivary glands, following Painter's method. She found that the whole region occupies only a portion, about 3/4, of one chromatic ring or node (the second) as seen in this material. (In the normal chromosome this ring sometimes appears double, but is not further resolvable by ordinary cytological methods.) Estimating the proportion which the region in question forms of the total material present in all the chromatic rings, we can thus already arrive at a tentative approximation of the number of genes in the chromatin; it is of the order of a few (ca. 5–10) thousand. This agrees with some previous estimates of the senior author, using totally different methods.

The differences in position of the different breaks were also discerned cytologically by Prokofyeva, pieces of obviously different thickness having been taken off the second ring in the different cases (see Fig. 1). The relative amount removed in each case corresponded with that to be expected on the basis of the genetic findings, as indicated in the schematic.
diagram (Fig. 2) herewith presented of the region in question (the end of the X-chromosome which is normally free). Even the difference in position of the two breaks (\(y^{3P}\) and \(sc^8\)) that were separated by the space of only two genes was visible, though nearly on the limit of microscopic resolution (being of the order of \(1/10\) of a micron). Genetic and cytological maps agreed throughout, and were linear. On the basis of these results it is also seen that the very thin rings found in various parts of the chromatin (notably in the fourth chromosome) turn out to be of about the same size as that which we have above deduced to correspond to an individual gene.

The results then show that at least the regions of the chromatic nodes ("rings" or "bands" of the chromosomes of the salivary glands)
contain genes (we leave still open the composition of the non-staining internodes) and that some at least of these nodes contain clusters of genes rather than individual genes. Since crossing-over is known to occur between yellow and scute, which are both in the cluster here studied, it is clear that the internodal regions are not to be identified with necessary positions of crossing-over. As yet, their function remains unexplained. According to the theory of Koltzoff8 (see also Alverdes9,10), which we accept, the nodes or rings of the salivary gland chromosomes represent chromomeres, inasmuch as an entire chromosome in a salivary gland cell is a hollow cylindrical bundle of uncoiled and parallel-lying chromonemata, formed by the repeated division and conjugation of the single chromonemata in two original homologous chromosomes, and since homologous chromomeres of all the chromomemata of a bundle would lie apposed, they would give the appearance of rings or cross-striations (cf. the structure of muscle fibres). Transferring then our conclusions from terms of nodes to terms of chromomeres, we see that the chromomeres contain in some cases at least whole groups or clusters of genes, the individual members of which are linearly arranged and are separable from one another both by breakage and by crossing-over. The diagram based upon this conception is shown in figure 3.

This conclusion seems at first contrary to the conclusion reached by Belling11 regarding the one-to-one correspondence between genes and chromomeres in certain flowering plants, but it is possible that the genes are more regularly spaced and isolated in the latter, corresponding more nearly with the smaller chromomeres found in some parts of the chromatin of Drosophila (e.g., in chromosome IV) and that finer optical methods (e.g., the use of ultra-violet light) might resolve the larger chromomeres of Drosophila into their gene constituents. Nevertheless, it must be recognized that in Drosophila the genes are not evenly distributed as Belling had pictured for flowering plants, and that most of the larger objects which appear as single chromomeres by ordinary methods really contain more than one gene, while the smallest chromomeres probably contain but one gene.

The genes of a cluster are capable of forming new clusters by synthesis, for cases were found in which a fragment broken off from one node (cluster) became attached in another region of the chromatin in such a way as to become directly joined onto a portion of another node (cluster), to form a new compound node. It should further be noted that this process of breakage can occur simultaneously at two different points within the limits of the very same node, with the deletion of the tiny region between the two points of breakage and its insertion into another region of the chromatin; this has been determined both genetically and cytologically (case of scute 19). Thus we see that even though a few genes are removed from the as-
associate genes on either side of them, they can still maintain themselves and reproduce in their new surroundings, and are in that sense independent of one another. Moreover, the genes in the one-gene chromomerers seem normally to be comparatively isolated (unless the internodes, too, contain genes).

The above work indicates the potentially discontinuous character of the hereditary material, in that it is divisible into definite blocks capable of self-propagation in any new arrangement, and it also indicates that this divisibility has certain limits of size. On the other hand, studies of the phaenotypic effects produced in the presence of these rearrangements have brought us strong evidence that the genes are, from the point of view of their functions in determining the characters of the organism, not discontinuous, in that neighboring genes enter into special relations with one another, with the resultant formation of a gene-system of a specific pattern, the
joint functioning of which, as such, is necessary for the normal development and maintenance of the somatic characters.

Several years ago Muller had found that changes in gene arrangement were usually accompanied by changes in the phaenotypic effects of genes lying near the points of rearrangement, and suggested\textsuperscript{1,15,18} that the general explanation of this result might lie in localized influences between progenitor genes, such as Sturtevant\textsuperscript{14} had already demonstrated in the special case of the Bar gene, but, as Muller pointed out\textsuperscript{1,15,18} it was also conceivable that the results might be due to mutations or losses of genes occurring concomitantly with, and caused by the same disturbances as, the chromosome breakage. Dobzhansky and Sturtevant\textsuperscript{16} later showed that phaenotypic changes accompanying breakage could be demonstrated rather regularly through the weakenings of dominance of genes near breaks in small fragments of active chromatin. Frequency of effect, however, might merely indicate the sensitivity of loci to the transmuting effect of the local process that simultaneously causes the break. Dubinin and Sidoroff\textsuperscript{17} also have recently shown that in the case of the locus of "cubitus interruptus" in chromosome IV, when there is a change in the gene arrangement near-by (translocation), an alteration frequently occurs, in the mode of expression of the originally normal gene. They find that this change is visible only as a weakening of dominance, and since this sort of change has not hitherto been detected very often among "gene mutations," they bring it forward as evidence that ordinary gene mutation has not occurred here, but that it is the change in gene progenitories which must be responsible for the effect observed. This argument, however, meets with difficulties in view of the mode of expression of such cases of gene mutation as those of "nick" in connection with vestigial, found by Bridges,\textsuperscript{18} of "vortex" in connection with "dumpy," by Muller,\textsuperscript{19} of "ebony 12" in connection with "ebony," by Stern,\textsuperscript{20} of "spineless" in connection with "aristopedia," by Sturtevant,\textsuperscript{21} and of "not-white" in connection with "white," by Muller\textsuperscript{22}; these cases are necessarily difficult to detect.

However that may be, we have now found in our present work crucial evidence from another direction for the position effect hypothesis. The scute character, the pattern of which is usually so different in the case of different allelomorphs, is in general affected if a break and rearrangement occur near the scute locus, but it is affected in all sorts of different ways in the case of different rearrangements. When, however, as in one case observed in our present work, practically the same rearrangement recurs, we find that practically the same phaenotypic change also has been produced (cases of scute 4 and scute L8). That the very case of such a rare phenomenon—the recurrence of phaenotypically the same scute mutation—should be accompanied by this other still rarer phenomenon—the recurrence of a case of double breakage in almost the same two positions—is
a virtual proof that the nature of the phaenotypic change was dependent upon the nature of the rearrangement.

In view of the fact that different phaenotypic changes of scute accompany different rearrangements near this locus, and in view of the essentially similar phenomena observed at other loci, we may then arrive at the broad conclusion that, in general, apparent mutations accompanying breakage are really resultants of the change in gene propinquities (position effect). We have found, moreover, that some "mutations" result from minute rearrangements (e.g., scute 19), and it is evident that, when these rearrangements are inversions, they must seem like "point mutations" so far as ordinary genetic tests are concerned. Thus the occurrence of a real intragenic mutation in any individual case suddenly becomes a matter very difficult of proof, although irrefutable general arguments for the general existence of such mutations still remain.

Most scute mutations, be it noted, are accompanied by some rearrangements, although the point of rearrangement lies outside of the scute gene itself, and can be several genes removed from the latter, and therefore the phaenotypic changes in these cases are not results of the loss or mutation of groups of sub-genes, as had formerly been hypothesized. The influence of gene propinquities no doubt is exerted according to an orderly system, in which certain neighbor genes play more important rôles than others in the production of given effects, so that their removal and the bringing near of other specific genes have definite effects on the characters in question.

In general, the influence, although it reaches across a number of genes, extends perceptibly over only a small distance, fading out at larger distances. We cannot, however, identify the range of the position effect with the node (chromomere or gene cluster), as an otherwise rather prophetic article of Brink23 would lead us to do. For Offermann and Muller, who have confirmed Dubinin and Sidoroff's findings concerning the weakening of dominance of the normal allelomorph of "cubitus interruptus" in translocations involving the fourth chromosome, conclude, on the basis of their own work, and contrary to Dubinin's conclusion, that the fourth chromosome itself is usually broken in these cases, and they find further that the effect sometimes extends also to the locus of eyeless. As it is very unlikely that the fourth chromosome should so often be broken in exactly the node of "cubitus interruptus" itself, or that eyeless should lie in the same node—especially since most nodes in this chromosome are of a size about corresponding with that above deduced for an individual gene—the conclusion follows that the position effect extends across a number of these small nodes. Hence the position effects are not blocked off into separate compartments, containing clusters of genes that are in this respect autonomous in relation to the other clusters, but, overlapping
from one cluster and from one region to another, they form in their entirety a continuum.

Whether the effect of propinquity is a direct one, of one gene on the structure or activity of the other gene, or an indirect one, due to a higher degree of interaction between locally more concentrated products of gene activity than between more distantly produced and either more diluted or changed products, cannot yet be stated. However that may be, we see that the basis of genetic determination of the characters of an organism cannot be stated completely by a mere listing of the individual genes which the organism contains; the arrangement of these genes also counts, and probably no rearrangement is without some effect. In other words, this arrangement is itself, in effect, genic in nature, and the genetic material of any one chromosome is in this functional sense a continuum.

Summary.—1. By a special genetic method, analysis has been made of a series of chromosome breaks in close proximity with one another. Selection of these breaks has been made by the aid of their position effects (see below). Analysis of the positions of each of the breaks with reference to each of the others has been made by obtaining recombinational individuals, containing the part of the chromosome to the left of one break and the part to the right of the other break, and the complementary combination, in the case of all possible combinations of two breaks.

2. The results of the analysis show that the breaks thus studied are subject to recurrence in the same or nearly the same positions and that the distances apart of those in sensibly different positions are of the order of size of individual genes.

3. Viable deficiencies of genes can thus be obtained, though most of the deficiencies are lethal.

4. The number of genes in a given region is thus found to be limited, and can eventually be counted. Since the size of the region is cytologically determinable in relation to the total size of the chromatin, the total number of genes also may be estimated. A preliminary estimate by means of this method places the order of magnitude of the number of genes at a few (ca. 5–10) thousand.

5. Differences of the order of size of individual genes are just discernible cytologically (with visible light) in the chromosomes of the salivary glands. The genes studied are found to lie in the region of the staining material or nodes, which (accepting Koltzoff’s explanation of them) represent chromomeres. A large chromomere, though not further resolvable by ordinary optical methods, is considerably larger than an individual gene, and contains a cluster of linearly arranged genes, between which crossing-over can occur. The small chromomeres, on the other hand, such as many of those in the fourth chromosome, are of approximately the size which the individual genes within the large chromomeres are found to be.
6. The genetic and cytological results were mutually consistent, and on the basis of them it was possible to make maps showing the positions of the genes, and breaks, within a portion of one large chromomere (band or node).

7. A section of the gene string which is sometimes so small as to comprise only a part of one chromomere can become detached from the chromatin on either side of it, and reattached in a different position. The genes thus have a far-reaching ability to maintain and reproduce themselves independently of their arrangement (except in so far as their life and reproduction are dependent upon that of the organism as a whole, and this in turn is influenced by the position effect mentioned below).

8. Where phaenotypic changes accompanying changes in the arrangement of genes are found to be very diverse (as in the case of the scute locus) our results show that an identical phaenotypic change accompanies an identical rearrangement, whereas different phaenotypic changes accompany different rearrangements. This constitutes crucial evidence that, in general, the apparent "mutational" changes accompanying gene rearrangements are really due to localized influences which propinquitous genes, or gene products, exert on one another (position effect). The position effect fades out at greater distances, but can extend over several genes, and over more than one chromomere in some cases. In this sense the genetic material is a continuum.

9. As some of these minute rearrangements must be practically indistinguishable, genetically, from intragenic mutations, the problem of how many and what supposed gene mutations are really only rearrangements assumes greater urgency.

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13 Muller, H. J., and Altenburg, E., Genet., 15, 283 (1930).
14 Sturtevant, A. H., Ibid., 10, 117 (1925).
16 Dobzhansky, Th., and Sturtevant, A. H., Ibid., 2, 45 (1932).
CRICETINE-LIKE RODENTS FROM THE SESPE EOCENE OF CALIFORNIA

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Introduction.—The Sespe deposits of southern California have yielded several cricetine-like rodent specimens. Rodent types other than those related to Paramys and its allies are rarely found in the Eocene of the North American continent. Hence, these specimens are not only of interest from the standpoint of adding new types to the Eocene fauna, but also in that they may eventually aid in the solution of the difficult and complex problem of rodent differentiation.

Eumysops simplex, n. gen. and n. sp.


Locality.—Sespe Uppermost Eocene, north of Simi Valley, Ventura County, California; Locality 150 C. I. T. Vert. Pale.

Generic Characters.—No antero-median cusp on $M_1$. Heel of $M_3$ not contracted posteriorly. Internal spur of hypostylid generally well developed. Protolophid uniting protoconid and metaconid; never disconnected from metaconid to form a pseudo-hypostylid spur as in posterior cheek-teeth of Eumys. Connection between hypostylid and protoconid weak, lacking in some specimens. No entoconid on $M_3$.

Specific Characters.—Metastylid somewhat less developed on $M_1$ than in Eumysops vetus. Metastylid not present on $M_2$. Connection between hypostylid and protoconid generally less well developed than in E. vetus, sometimes lacking.

Description.—No. 1778 is the only specimen of Eumysops which shows much of the ramus. The masseteric fossa terminates under the first molar. The masseter lateralis ridge is relatively strong, the masseter medialis ridge relatively weak. The latter characters are present in Eumys