COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY

VOLUME XVI

GENES AND MUTATIONS

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FOREWORD

It is ten years since I took over the responsibility of organizing these Symposia; and it seemed appropriate to recognize this tenth anniversary by the selection of a symposium theme similar to that discussed in 1941 and of particular interest to me and my geneticist colleagues at Cold Spring Harbor. Once more a group of key research workers, active in the rapidly developing branch of genetics that seeks to analyze the mechanism of heredity, met to exchange results, and their reports reveal the striking progress made in the past decade.

The original problem of defining the unit of heredity, which almost fifty years ago was designated "the gene," has not yet been solved. In fact, the large body of information accumulated since 1941 has made geneticists less certain than ever about the physical properties of genes. Ten years ago they were visualized as fixed units with precise boundaries, strung along chromosomes like beads on a thread, very stable, and almost immune to external influences. Now, however, they are regarded as much more loosely defined parts of an aggregate, the chromosome, which in itself is a unit and reacts readily to certain changes in the environment. The apparent resistance of genetic factors to outside influences is not so much a reflection of genic stability as a result of the fact that conditions likely to affect a gene will also produce injurious changes in other parts of the cell, which in most cases will be lethal. Ten years ago, only X-rays and ultraviolet rays were known to induce changes in genes; but reports given at this year's Symposium have made it clear that such changes may also be brought about by a great many chemicals.

One of the most remarkable developments of these ten years concerns the organisms used in gene studies. In 1941 about thirty per cent of the Symposium papers reported research carried on with *Drosophila*, and only six per cent dealt with microorganisms; whereas this year only nine per cent of the papers relate to *Drosophila*, and about seventy per cent to microorganisms. This should not be interpreted as evidence that *Drosophila* research has declined. The directory of this year's "Drosophila Information Service" (DIS-25) shows that more research laboratories and workers are now using *Drosophila* than ever before. The entries reveal, however, that a considerable proportion of these workers are engaged in studies of population genetics. It appears, therefore, that the low representation of *Drosophila* research in this Symposium is due to a shift in interest among *Drosophila* workers rather than to decreased study of this material by geneticists.

One of the papers presented at the 1941 Symposium was "Image Formation by Electrons," by Dr. V. K. Zworykin. At that time the electron microscope had just been made available to science, and, because it offered promising possibilities for cytogenetic research, a competent review of the subject was considered desirable. This year Dr. L. E. Flory's paper, "The Television Microscope," was included in the Symposium, to call the attention of geneticists to another powerful new tool that may play an important role in cytogenetic research, particularly ultraviolet microscopy.

The meetings were held from the 7th through the 15th of June, and were attended by more than 300 participants. The expenses of the Symposium, especially those connected with foreign guests, were covered by a grant from the Carnegie Corporation of New York. The editor of the volume was Dr. Katherine Brehme Warren.

M. DEMEREC

LIST OF PREVIOUS VOLUMES

Volume I (1933) Surface Phenomena, 239 pp.
Volume II (1934) Aspects of Growth, 284 pp.
Volume III (1935) Photochemical Reactions, 359 pp.
Volume IV (1936) Excitation Phenomena, 376 pp.
Volume V (1937) Internal Secretions, 433 pp.
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THE THEORY OF THE GENE

CHROMOSOMES AND GENES

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I have been asked to report here upon my *present views* on the problem of "Chromosomes and Genes." I emphasize the words "present views" and explain the reason for this by the quotation of an autobiographical remark by Darwin: "I have steadily endeavoured to keep my mind free so as to give up any hypothesis, however much beloved, (and I can not resist forming one on every subject) as soon as facts are shown to be opposed to it."

I not only appropriate these lines, but also recommend them to those who think one can oppose new ideas not by better ones but by playing them down and even covering them with silence. Fortunately there is a cure for backward turned heads which in the long run always succeeds, namely more and more facts and honest discussion.

While thus introducing my report with a statement of willingness to learn, I must add that I strongly believe that my way of looking at the facts available today comes nearer to the truth than other interpretations; and I add with an understandable modicum of satisfaction that more and more workers in the field are beginning to direct their thoughts in a similar direction. As so many representatives of different, as well as similar, points of view are present here as speakers on the program, I feel entitled to restrict my presentation to my own views. I shall try to present these without entering into any detail which could be done only in a series of papers.

Any discussion of the subject will, in the end, turn to the problems of mutation as all our knowledge of the genetic material, in so far as it is not determined at the cytological level or is the result of biochemical analysis, is derived from the study of mutation. In classical genetics the gene is an extrapolation from the mutant locus. The existence of a linear, polarized organization of the chromosome is proven by the typical and strictly localized actions of mutant loci and their orderly recombination by means of crossover breaks. This insight led to the assumption of material units, the genes, individualized, atomistic but integrated in their action; able to change from their specific condition into other stable ones, the mutant genes (which includes multiple alleles and isoalleles). The appearance of a mutation manifests itself by means of a changed effect based upon a strictly localized action and suggests that at the same location another entity, the original gene, had been present. What seems to me the best elaboration of this concept is contained in Beadle's one gene—one enzyme idea: a mutant locus is shown to be connected with the failure of production of a specific enzyme for a definite step in a synthesis; this fact is supposed to prove that the original gene is responsible for the production of the respective enzyme. This is what I called the extrapolation from the mutant action to the existence of the original gene. I am aware that Beadle himself has since demonstrated that things are not so simple and has generously acknowledged this. But I am not concerned here with the correctness of the specific idea but with the fact that it so excellently represents the logic at the basis of the theory of the gene.

The last two decades have brought to light a considerable body of facts which have led to a reconsideration of the basic tenets of classical genetics. It is obvious that this does not mean that the body of genetic facts could not be presented as before by the use of the notion of the gene. It means only that it has to be considered that this notion may be too simple and primitive. Repeating a comparison which I have used formerly, the situation is similar to that in recent chemistry. Practically all of standard chemistry may be described in terms of the old concept of valences, symbolized as one or more dashes, and the average chemist need never go beyond this level. Is it therefore wrong to inquire into the meaning of the dashes and to show that valence electrons and the laws of quantum mechanics are needed for an understanding?

In a discussion of the subject of mutation, the first important group of facts is that concerned with the radiation effect upon chromosome breakage. It is a discovery of major importance that

chromosome breaks, induced by radiation, follow the same law of proportionality to dosage as do visible point mutants or lethals of whatever kind. So far as I can see this fact can be explained in only two alternative ways. According to the first hypothesis a hit may produce either a mutation or a chromosomal break depending upon whether genic or nongenic chromosomal material is hit. The second hypothesis implies that the result of a hit is always the same; namely the production of an intrachromosomal rearrangement. With the exception of terminal deficiencies, of course, two breaks are needed for a rearrangement. But as it is known that the dosage law applies also to two closely existing visible breaks there is no objection in principle to the production, by a single hit, of two breaks on the submicroscopic level.

My personal predilection is for the latter alternative. It does not require that two different processes occur under the same treatment with the same numerical results. Actually the first alternative would never have been considered if it were not for the presupposed assumption of the gene molecule. Furthermore, looking at the radiation effects as a whole, we may find two breaks at any conceivable distance, from almost the entire chromosome length down to a single band of a salivary chromosome, and we may infer that single breaks may occur and heal, and therefore remain unobserved. (See illustrations of a single band deficiency at the arc locus in *Drosophila* in Goldschmidt, 1945, and of what is most probably a single band inversion in Goldschmidt and Hannah, 1944.) The logical consequence is that rearrangements within a single band, i.e. invisible ones, representing the so-called point mutations should also exist. I add that the only definition of point mutation is its invisibility in the salivary chromosomes, i.e., a completely arbitrary delimitation based only upon the limits of the light microscope.

Those unwilling to accept the logical conclusion that point mutants are only the effects of rearrangements on a submicroscopic scale might ask whether any actual observations exist which point to happenings within the smallest visible structure, the single salivary chromosome band. Kodani and myself (1943) found that frequently the tip of one of the synapsed chromosomes seemed to be missing and in this case the deficient side ended with one rather thick band, thicker than its partner. Correcting the original interpretation, Kodani (1947) proved that a deficiency was not involved but that the tip can contract so as to shorten for the distance of a number of bands all of which unite into a single one. If this is possible at the limits of optical resolution, there can be no doubt that an invisible subdivision within a band is possible and even probable. Strong support for such ideas is also available from genetic experimentation. Years ago Gottschewski (1937) found that a Notch deficiency which had appeared in my experiments was genetically a deficiency while the salivary chromosomes were normal. Many similar cases, all at the Notch locus, have since been described by Demerec (1943) and Barigozzi (1942). The explanation given by all these researchers was that the normal genes had not been deleted but only inactivated, an interpretation which I consider to be born out of embarrassment. There are only three ways known in which a heterozygous recessive mutant can control the phenotype: 1) in the presence of appropriate dominance modifiers—this is excluded in the present case; 2) in hemizygous condition opposite a deficiency —even assuming a submicroscopic deficiency this is highly improbable, because in some of these cases such invisible deficiencies would have to be assumed for a number of loci; 3) in the presence of a rearrangement near the locus or loci in question in the homologous chromosome, a so-called position effect. Only this third case fits the facts, especially if we think of McClintock's invisible transpositions of loci and of the fact that one rearrangement break can produce a position effect for quite a distance. Thus, an invisible rearrangement is the best explanation for these cases and simultaneously an indication of the existence of rearrangements below the microscopic level.

There are certain other facts pointing in the same direction, besides McClintock's work which she is reporting here herself. I described a case in *Drosophila* (Goldschmidt, 1948) in which the genetic analysis demanded a transposition with resulting deficiency after crossing over between the two points. But the salivary chromosomes were completely normal. Altogether, I cannot see any objection to the assumption that mutants are submicroscopic rearrangements, which would also include deficienties, a subject which I expect Stadler to touch during this Symposium.

The most important arguments against the classic concept of the particulate and separate gene are of course derived from the phenomenon of position effect. This effect was confined till yesterday to *Drosophila*, and a few cases of unequal value in *Oenothera*, *Nicotiana* and *Zea*.

In view of the facts that not only are all typical genetic phenomena identical in both kingdoms, but also that the dosage law for chromosome breaks induced by radiation is as true for plants as for animals, one had to expect that position effect would also be a phenomenon common to both kingdoms. Today, after McClintock's brilliant work, it has become one of the most common genetical phenomena in plants, if one is permitted to assume that all the numerous cases of so-called mutable genes have the same cytological basis!

Let us begin the discussion of this effect with the decisive fact which I tried to put in the foreground in all my former discussions of the subject (Goldschmidt, 1937, 1940; the last named paper contains all the details and quotations which have no place in the present review), but which, strangely enough, has not been emphasized properly in some recent discussions. It is the fact that in all important cases position effect means that a normal locus, near a rearrangement break, acts as if it had mutated to its typical mutant action. A break near the normal locus of yellow makes the $+^{y}$ locus, whether in its old position or moved to another location by being included in the rearrangement, act as if it had mutated. The phenotypical effect is yellow in the hemizygote, in the homozygote of the rearrangement in the duplex condition and in the compound with standard yellow mutants. The reason why this central fact has frequently been hidden is probably that the first discovered and named position effect was a different phenomenon if not looked at in the light of present day knowledge. Two so-called Bar-genes in one chromosome had a different quantitative effect from that when located in both chromosomes respectively. If a point mutant for Bar eyes were known in this region, the single Bar effect would have been recognized at a later time as a position effect caused by the duplication break, and the difference of action of a triplication in one chromosome as the additional action of another break, which changes the normal order once again. In other words, the relevant point would not have been the presence of Bar in one or both chromosomes, but rather a single homozygous position effect in one case *versus* two heterozygous ones in the other case.

A second reason why the decisive point seems missing in many discussions is that a large number of obvious dominant position effects is hidden behind such terminology as: dominant mutant inseparable from inversion N or translocation M. It is true that in most of these cases a nearby locus connected with the phenotype of the position effect is not known. The fact that a locus is sometimes known to be involved, i.e., brown for the Plum effect in *Drosophila*, indicates that in other instances the identification of such a locus has escaped us. A third reason is the occurrence in some cases of a mosaic position effect which has, most artificially, been made out as a completely different thing. In fact this is not different, in principle, from the typical effect, namely action of the break identical with that of a nearby mutant locus. The difference from the simple position effect is one of phenotypic action in development. The penetrance in the individual cells or group of cells affected is variable, in many cases around a threshold producing an all or none effect, the cellular mosaic. This is comparable to varying penetrance on the right or left side of an individual or between numbers of a sibship in mutants with incomplete penetrance. (In some cases e.g. at the white locus it is not an all or none effect but an effect of varying intensity, visible as many intermediate conditions—like multiple allelic effects—between the two extremes.

Thus the really important fact about position effect is, apart from secondary variants, that a breakage of the chromosome makes a nearby and unchanged locus act as if it had mutated to one of its allelic forms. This leads at once to the question whether the break itself may be responsible for the effect, in which case the production of pasted together ends with a scar substance in between may be visualized. Or it may be that the changes in the serial structural order of the chromosomes are the real cause. We shall return to this issue later, after a few more facts of importance have been mentioned.

One is derived from Beadle's well-known work on sticky chromosomes in maize (1932), where a genetically controlled property of the chromosomes, stickiness, leads to a great increase in both chromosomal rearrangements and point mutations. It does not seem possible to explain this fact except with the assumption that point-mutants are also invisible rearrangements with a position effect. There is, further, Sturtevant's report (1939) that after crossing of two *Drosophila* species both rearrangements and point mutants increased considerably, at least in one case. As the chromosomes in this cross show considerable pattern differences leading to faulty synapsis, the same conclusions as before can hardly be avoided.

Some time ago Demerec (1941), when discussing mutable loci, stated that this phenomenon is after all not so surprising, because all transitions from little to more and more frequent mutability are to be found in different strains of one organism or in different organisms, and mutable loci therefore only mean one end of such a series of frequencies. I have no doubt that this argumentation is correct, though in a different sense than originally intended. After McClintock's proof in *Zea* that mutable loci are actually position effects produced by genetically controlled and repeating transpositions and translocations (leaving out here all further details), the just mentioned statement by Demerec would now have to read: "All mutants, whatever their frequency, place, cytological visibility as rearrangements, or non-visibility are position effects." Thus I feel more confident than ever that my own former argumentations moved along the right path. If it would have happened by chance that all the facts just discussed had been discovered in the early days of Mendelism, the theory of the corpuscular gene, invented in pre-Mendelian times by DeVries and conceived in terms of serial chromosomal structure by Roux and Weismann would never have taken hold of genetics.

We may conclude this part of our discussion by emphasizing that there is not a single type of mutant action known which does not occur also as position effect. There are dominant and recessive position effects. Dominant effects may be homozygous lethal or not (Bar, Plum, Dichaete in *Drosophila*). Recessive position effects are frequently seen when the break in a normal chromosome is in compound with the proper recessive mutant or, where possible, as homozygous rearrangements. Varying dominance conditions, paralleling situations in multiple alleles, and even change in dominance (the cubitus interruptus work of Dubinin, 1936, and Stern *et al.*, 1943, 1944, 1946) may be found when different rearrangements are tested in compounds with recessive mutants. Just as in the case of so-called point mutants such different phenotypic effects in different compounds are specific features of genic action involving degrees of action and potencies, as well as threshold effects, in short, well-known phenomena of physiological genetics. There are further position effects acting as modifiers, enhancers of penetrance and dominance, as described by Gardner (1942), Goldschmidt and Gardner (1942) and Goldschmidt (1945). Position effects acting like mutant loci controlling mosaic development are known, both with regard to purely embryological segregation and to production of chromosomal deletions (Schultz, 1947; McClintock, 1950). Finally position effects may be reversed (Gruneberg, 1947; Hinton, 1949) like reverse mutations. Thus a difference between position effects and point mutations has still to be discovered.

We return now for a moment to the problem touched upon before, whether the position effect is produced by the break itself or by the accompanying rearrangement. One might claim, and has claimed, that irradiation must frequently produce single breaks which are healed at once. These might be the invisible point mutations. Under this assumption, then, the blocking of the chromosomal continuity alone would produce the effect upon neighboring loci. The known facts are not in favor of this assumption. If it were true one should frequently find neighboring loci mutating together, namely, if the invisible and healed break falls between them. As far as I know the only case in which it has been shown that two loci mutate frequently simultaneously applies to loci in different chromosomes (arc in the second and silver in the first chromosome, Goldschmidt, 1947). One should further find point mutants near the break of a terminal deletion and in cases of whatever fragmentation of chromosomes, assuming that the interruption itself, not the healing process is responsible. The only case known to me which would support such an interpretation is Sutton's deficiency left of the yellow locus, which, however, may also be interpreted differently. A serious objection is also the fact that crossover breaks do not produce position effects, though I am ready to concede that we have no knowledge whether crossover breaks and rearrangement breaks are actually the same thing. Unfortunately we are still without any real knowledge of what happens in the chromosome during crossing over. Thus it seems that the real cause of position effect as well as point mutation is the change in the serial order of the intimate organization of the chromosome.

Do we have any more information about the meaning of this serial order? An interesting and well-integrated group of facts leads to the next step in our analysis. For a number of loci in *Drosophila*, series of multiple alleles are known, some of which turned out to be point mutations, others position effects of inversions and translocations. In some cases, the bands in the salivary chromosomes can be found by the deficiency method which are supposed to contain the locus of the point mutation. Furthermore, the breaks are known which produce the allelic position

effects. In some of these cases, e.g. yellow or scute, a number of breaks on both sides of the assumed locus produce the position effect so that a position effect segment, containing two to six bands at least is delineated. (For details see Demerec, 1943, and Goldschmidt, 1944.) This means, if we adhere to the facts discussed above, that whatever happens within this segment produces the mutant effect typical for the so-called locus. In detail, the effect is produced as well if the locus remains *in situ* but part of the segment is removed to another location in the same or in a different chromosome; or if part of the segment including the locus is removed to another position; or if part of the segment not containing the locus is deleted; or even if the locus itself is deleted (yellow deficiency) presumably with a part of the segment remaining; or finally if a submicroscopic change within the locus itself occurs, the point mutation. A corollary of these purely descriptive facts would be that submicroscopic changes, e.g. point mutations, could occur in all bands of a section, which involves the very heretic idea that there might be as many loci of point mutations as bands in a section. We shall later mention the facts which support this idea and point out here only, without further discussion, that the same facts have also been described in terms of genes and subgenes (Serebrowsky 1930; Raffel and Muller, 1940).

There are a few more interesting facts connected with these segments of mutant action. The most remarkable one is that these segments may be overlapping as found by both Demerec (1943) and myself (1944). This means that in one case a break to the right of a definite band within the segment produces the mutant effect of a locus in the segment to the left and vice versa. It is difficult to explain this fact. It requires some tapering off of the segmental structure with overlap so that the break hits parts of two segments with the possibility of position effects in both (for which we gave as an example yellow and achaete, Goldschmidt, 1945) or in other words, the overlap effect. Certainly more facts are needed for the evaluation of such cases.

The other remarkable fact, found by Demerec (1940), is that a break may cause the mutant effect to appear in quite a series of loci and therefore segments up to 40 and more bands away from the break. This happens only, as far as present knowledge goes, if chromocentric heterochromatin is being intercalated between the break and the rest of the chromosome. This shows that the mutant action of an unchanged locus is not only produced by happenings within a segment but also by a specific distant discontinuity of the chromatic order of the chromosome. This fact induces us to ask how the sections of mutant action are integrated into the whole chromosome as parts of a major unit of action.

Before we take up this problem we must return once more to the segments of mutant action. Earlier we arrived at the conclusion that any band contained in such a section of the salivary chromosome could be the seat of identical, allelic point mutants, assumed to be based upon submicroscopic rearrangements. There is a group of facts which in my opinion find their proper explanation within the framework of these ideas. I mean the so-called repeats as studied by Lewis (1945), Green and Green (1949), Laughnan (1949), Raffel and Mulller (1940), Komai (1950), Komai and Takaku (1949). The decisive facts are these: two or more mutants are found which behave like multiple alleles. Both produce if homozygous and recessive a definite effect, similar but slightly different for each. (A similar situation has been described by Bonner, 1950, in *Neurospora*, where, however, all three loci are needed for the biochemical effect. The work of Stadler and Fogel, 1945, probably belongs here too.) In a compound a comparable effect results thus producing the semblance of a series of multiple alleles. But since actually crossing over takes place between the two (or three) loci it is said that we are dealing with a number of genes with pseudoallelic effect. It is assumed that these originated as repeats from one primary gene, which changed their action somewhat but retained the property of allelism. (In addition each may have its own multiple alleles.) Retaining the property of allelism means that the presence of any one in both homologous chromosomes acts like a homozygote while any number in only one homologue has no effect. Whereas ordinarily only mutants of the same locus act like alleles, here all three loci—in Green's case—behave as alleles if located in any arrangement in both chromosomes. The situation, therefore, has been described as a position effect in the old sense of Sturtevant, namely a difference of genic effect depending upon whether the genes are located in one or both chromosomes. This comparison with the Bar duplication is actually based on a very superficial resemblance. We have already emphasized that in the Bar case it is not the location of mutant loci in one or both chromosomes which makes the difference but the presence of one homozygous break *versus* two heterozygous

ones, or, expressed differently, a single homozygous position effect *versus* two heterozygous but dominant ones.

If we now turn our minds back to the former discussion of the chromosomal segments, within which invisible point mutants, rearrangement breaks and even deficiencies will all produce similar effects, all of them acting like multiple alleles, the facts just discussed fit completely into this concept without requiring further explanation or assumptions, though adding the interesting point that crossing over occurs within these segments. One consequence of this interpretation is that one should expect to find instances of relatively large segments with many multiple alleles (and position effects) between which crossing over is possible. I consider it quite likely that the blood-group situation in cattle studied by Irwin and students, with its 80 and more multiple allelic combinations, presumably based upon crossing over between a very high number of points with similar action, is an example of this kind. In the most recent discussion by Stormont *et al.* (1951) this idea is not considered though a rather similar one is presented involving the notion of subgenes, a notion which after all means only a different terminology from ours. This however, is not accepted by Stormont *et al.* who are inclined to a serological interpretation involving different actions of the same locus.

We are now ready to take up the problem of how the chromosomal segments are integrated into higher units of action within the chromosome to which the distance action of heterochromatic breaks has already pointed. I realize how difficult the visualization of this and the following points is for the geneticist who is accustomed to think only in terms of genes. Let us try to make the underlying ideas clearer by the use of a simile, which like all such comparisons should not be worked to death but be considered only as a help towards a visualization. If we study the action of light waves upon an organism we may find definite actions of ultraviolet light of one single wave length, which we compare to the action of a single genetic locus. We might then find actions which are essentially the same over a number of wave lengths, say within the ultraviolet. Other actions may be typical for the entire ultraviolet or other part of the spectrum and finally some light effects may be the same over whole sections, e.g. the yellow-green sections of the spectrum. In this simile, we compare the single wave length to the mutant locus, a few wave lengths to a section and the other groups to larger and larger sections of a chromosome.

It is a fact that a mutant locus generally produces its typical effect not only if located at its proper place but also if it is inserted within a chromosomal segment somewhere else in the chromosome set. (But it should be added that a thorough exploration of this point is still missing.) One might be inclined to conclude from this that it is only the locus, the gene, which has its independent action. The simile is meant to show that independent of the local action of a locus an overall action of a chromosomal segment of different length is also imaginable. For example, a definite mutant locus in the third chromosome in *Drosophila* at point 58.5 may change the normal development of the cephalic disk into the aristapedia type. But this locus may be a part of a larger section of the chromosome which, as a whole and not by additive action of its parts, is in control of the general developmental features of imaginal disks, while individual points in this section, the loci, if mutated, disturb this action in a definite though related way for the different loci. To be more specific: in this rather large section the loci bithorax and proboscipedia are found. We do not assume that these loci, if not mutated, control individually the development of the respective discs so as to prevent them from sidesteps while the mutant locus produces that sidestep. We do assume, however, that the whole intact section controls certain parts of normal development of the imaginal discs, *ceteris paribus;* but that a disturbance of this action at individual points leads to similar changes—all are homoeotic mutants—but which are in detail different for the loci.

I have chosen this example in anticipation of a group of facts which may now be discussed as a factual contribution to support such conclusions. The number of cases is increasing in which it turns out that mutants with a generally similar though individually specific effect are located within a more or less extended section of a chromosome. The just mentioned homoeotic mutants are one case, Dunn and Caspari's (1945) *T*-factors for mouse development another, Nabours' (1950) proof that all the many mutants affecting the color pattern of the grouse locust are located in a relatively small section of one chromosome is another instance. Other cases belonging to this group have recently been assembled by Komai (1950). Recently Pontecorvo (1950) and students (Roper, 1950) have attacked this problem in microorganisms and the first case of three closely linked loci affecting different steps of biotin synthesis has been discovered. All this tends to give support

to this part of our ideas on chromosomal configuration and action.

At this point the difficult question should be raised as to whether the general idea could be carried to its logical end by assuming that also a generalized action of an entire chromosome, independent of the partial actions of smaller segments down to the mutant locus is possible. It is very difficult to prove or disprove this conclusion because experiments which may be relevant always involve genic balance phenomena and thus cannot be decisive. There is no doubt that the chromosome is some kind of a unit, a fact for which the best proof is seen in the remarkable features of the chromosome arms in *Drosophila* species. The individual basic chromosomes—called the arms A, B, C, etc.—remain constant as such though they may enter different configurations and certainly become different in their intimate structure thus revealing some kind of important function of the whole. Another fact of this type is that 350 species of short-horn grasshoppers have the same chromosome number and configuration. Certainly this shows up the chromosome as some kind of unit though it is difficult to decide whether this means a genetic unit, a hypermolecule of individualized action, or only a mechanical unit of mitotic manoeuvering without genetic unity. A critic could quote the fact that the presence of broken and abnormally reconstituted chromosomes, including fragments with spindle fibers, even small ones, do not seem to change the genetic effects of the genome. But a comparative study might reveal differences in viability which is a definite genetic effect with unanalyzed detail. Another fact which can be explained on the basis of chromosomal action (though not necessarily) is the frequently found lethality of homozygous inversions and translocations which might be due to a breakage effect (position effect) or to the absence of two properly acting integrated chromosomes. At this point it should be mentioned that Mather (1946, 1948) has joined me in the views presented here. He expresses himself somewhat differently, calling the sections of genetic action of varying size from one locus to a whole chromosome "fields of cooperation," which may be varying during the life cycle of the organism. [The diagram which he presents (1948, p. 213) is the same which I have frequently used to explain my views]. This seems to me a rather fortunate term if the stress is on "field," not on "cooperation," the two words being, in my opinion, antagonistic to each other and the idea not being a cooperation of subunits but a field of action of different extension as the case may be.

This now leads to a further step in the analysis, actually already contained in the last discussion. None of the conclusions which I have presented during the past 15 years has been considered more shocking (even a worthless play of words, if not outright crazy) than the conclusion that the normal gene is a possible but not necessary extrapolation from the mutant locus. It is obvious that the only information we have about the gene is the mutant locus (with all its alleles): because mutant loci are proven to exist it is assumed that the not mutated locus has its share in the control of the normal character. The logical situation is again best expressed in the one gene—one enzyme theory. Because a certain step in the syuthesis of a genetically controlled product is prevented to occur by the mutant, presumably via the absence of the specific enzyme for that step, it is concluded that a normal gene exists which controls the production of this enzyme. The existence of position effect already shows that this type of conclusion is not necessary. The mutant effect is here produced by a change in the order or arrangement of the chromosomal parts, none of which is missing. The same type of reasoning as before would lead to the conclusion that the normal order is required for the typical effect, which does not necessarily imply the existence of a normal gene. The meaning of this may be better visualized if we introduce again two similes. The first has only a vague resemblance to what it is supposed to clarify. If the A-string on a violin is stopped an inch from the end the tone C is produced. Something has been done to a locus in the string, it has been changed in regard to its function. But nobody would conclude that there is a C-body at that point. A better simile can be derived by comparing the chromosome or its parts to a molecule. The molecule reacts in a definite way. If at one point of the molecule a different radical is substituted, say by methylation or amination, the resulting molecule may have completely different reactions. Can we conclude that the point in the molecule at which the substitution was made is in control of the standard reaction of the molecule? I should say that in either case, before and after substitution, the whole molecule reacts, not its loci.

If we look for an actual proof for the extrapolation from the mutant locus upon the normal

gene only one group of facts can be seriously contemplated. This is the covering action of a duplication, which may even be attached to a different chromosome. It is true that as a rule the duplicated locus (which includes always quite a segment) covers two mutant loci or one plus a deficiency. But it does not always do so. In the best studied case (cubitus interruptus, Stern *et al.*, 1943, 1944, 1946) a variety of conditions have been found. Cases exist in which two recessives are not covered by the duplicated normal locus; and I found a case where a duplication (for the silver locus) covered some silver alleles, but did not cover another (Goldschmidt, 1945). A similar situation was described by Muller (1935). Thus I am not inclined to consider this proof for the reality of the gene as binding.

This report upon my ideas concerning mutant loci and chromosomes cannot be finished without a few words on a subject which is coming more and more to the fore, the meaning of heterochromatin in the structure and function of the chromosome, to which we had to allude already in connection with the mosaic position effect near heterochromatic breaks. I do not need to go into details as I have discussed various aspects of the problem of heterochromatin only recently (Goldschmidt, Hannah, and Piternick, 1951). In connection with the present problem, the following seems to be significant. The bulk of the heterochromatin, in *Drosophila* at least, is the chromocentric heterochromatin which is easily dissociated from the chromosomes. Experiments of Mather (1944), L. V. Morgan (1947) and ourselves (Goldschmidt *et al.*, 1951) have shown that this substance has a generalized genetic effect insofar as a change in its quantity affects the action of many loci in a quantitative way e.g. by shifting dominance and penetrance. It is this same chromocentric heterochromatin which is connected with the mosaic position effect which thus should be interpreted as an ordinary position effect of a break plus a variable effect, in relation to a threshold, upon the degree of action within different cells; in other words, penetrance within the parts of the affected organs. The very generalized effects of sections of the Y-chromosome in *Drosophila* (sterility effect) or in *Melandrium* (general effects in the sphere of sexual differentiation) fall in line with the facts. White (1950) calls this the possession by the heterochromatin of a residual genetic function.

Future interest will, I think, be centered more upon the intercalary heterochromatin which is an integral part of the chromosomal structure with specific chemical features (the allocycly) and also physical features (breakability). In our recent work, we had tried to show—though the evidence is only of the indirect type—that these sections are the seat of genetic changes affecting early developmental processes. This is revealed in the form of groups of similar effects upon the entire organization based upon loci distributed all over the chromosomes, like the Minute effect and the podoptera effect. In the latter case a kind of pseudoallelism between all loci points to the general similarity of all the effects. If the interpretation turns out to be correct, the heterochromatic intercalary sections would assume an intermediate position between the euchromatic breakage sections discussed above and the generalized chromocentric heterochromatin. These problems and the underlying facts are only emerging and we are inclined to anticipate here a rich field of future research. This conclusion agrees also with McClintock's recent work in which an interesting relation between heterochromatin and chromosome breakage leading to mutable loci is described. Whether it is possible to explain the facts of mosaic position effect in *Drosophila* in the same terms as the maize mosaics is a question which the future will have to decide. I personally doubt it, assuming as mentioned before, that a purely developmental feature in *Drosophila*, namely penetrance varying around a threshold, is involved. One of the facts in favor of this interpretation is the occurrence of multiple allelic effects within the mosaic. (See discussion in Goldschmidt *et al.*, 1951.)

This is not the place to discuss evolution. But as it was said once (by Muller) that our point of view would lead to difficulties in explaining evolution, I should like to insert a few words on this subject. One of the few positive facts which we know concerning chromosomal structure and evolution is that in *Drosophila* species the intimate structure of the chromosomes becomes increasingly different with taxonomic distance (though not exactly proportionally at the lower level of distance). Between relatively distant species the chromosome structure appears completely scrambled and if the generic level is reached there may not be a shadow of resemblance left. In my opinion, only two possibilities exist: either this change in intrachromosomal architecture has no meaning, is a chance concomitant of other evolutionary processes, or it is an essential part of evolutionary diversification. The later means that the repatterning of

the chromosome creates a different system of genetic action based upon the new order of the polarized constituents of the chromosome. Such a happening would be a logical consequence of our general views, a consequence with an importance reaching far beyond that of possible changes by gene recombination. To realize this, one has indeed to forget to think in terms of genes. But even those who refuse to do this find themselves in a position to be forced to consider at least some such ideas though trying to keep strictly within the gene concept. Thus Dobzhansky writes in his book (1941 p. 85) "Divergence of evolutionary lines does not involve merely increase of the number of gene differences, it probably involves also changes of the developmental functions of the genes and a gradual increase of the differences between the structures of the genes which had been identical or similar in the ancestral forms."

Coming to the end of this report I return to the introduction in which I said that I was invited to describe my present views on the subject of chromosomes and genes. In doing so, I had to omit largely the details of the factual material upon which my conclusions are based and which can be found in former publications. I am fully aware that I have been dealing with a controversial subject which contains its attractions as well as its pitfalls. Rutherford said, "A well constructed theory is in some respects undoubtedly an artistic production." He could have added that, as such, it will necessarily contain many personal elements which cannot always be defined completely, nor enjoyed equally by everybody. There might even be found contradictions to which I should like to apply Unamuno's words (quoted from Schroedinger) "Si un hombre nunca se contradice, sera porque nunca dice nada" (If a man never contradicts himself the reason is that he never has anything to say). A theory trying to unify a vast and difficult field with innumerable details is certainly nothing static; it is a fleeting moment in an eternal flux, though there will always be men who regard their own pet ideas as unchangeable. For them, I quote Max Planck's description of the qualities of scientists: "Conscientiousness in the pursuits of important things, patience and the courage to stand up for his own convictions against anybody, even against his own former and different opinion." But the decisive point in forming generalizations is, in my opinion to look forward, not backward, and thus I am ending with a last quotation from D'Alembert (quoted from H. Margenau) "Allez en avant, la foi vous viendra" (March forward, the faith is bound to come).

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DISCUSSION

ORNSTEIN: This comment refers to Dr. Goldschmidt's view on the nature of *all* point mutations. The implication is that since the genesis of life there has been but one (or perhaps a few) chemical chromosomal substance which has had the potentiality of giving rise to all the genotypes of living organisms *without further chemical change* (other than the changes that it undergoes in autocatalysis and in being incorporated in "chromosomal units").

Now this does not seem too reasonable (even in the light of the remarkable properties of DNA) —and Dr. Goldschmidt, himself, has suggested that methylation or other types of substitution, for example, may be expected, at times, to give rise to mutations. How then can we presume to say, at this time, that *all* (or even most) point mutations are *either* of this latter type *or*, on the other hand, due to chromosomal rearrangements of the type associated with position effect.

PAPAZIAN: You mentioned briefly that crossing-over presented some difficulties to the concept of the gene that you have presented. I can perhaps imagine a mechanism of crossing-over that does not involve the breakage of chromatid strands. But even admitting this there seems to be a real difficulty in explaining the great precision of crossing-over and the absence of any position effects therefrom. Surely a genic unit defined as the smallest hereditary unit that cannot be resolved by crossing-over is real and useful even though it does not correspond to the physiological gene unit defined by its activity.

TAYLOR: I wish that I could say something in behalf of the recently deceased—the gene—but my findings may more nearly support the concepts of Dr. Goldschmidt. The last visible corpuscular unit of the classical cytologists which has been associated with the chromosome, the chromomere, has revealed itself as a manifestation of spiral structure. It appears to be formed by a chemical cross-bonding of certain adjacent gyres of a spiraled fiber with a corresponding loosening of the