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DUPLICATION OF CHROMOSOME PARTS AS A FACTOR IN EVOLUTION[1]

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To me the duty of addressing you this evening here in Boston is a particularly disconcerting one. My mind goes back unavoidably to that corresponding occasion twenty-four years ago, when those of us whom the gods favored had the privilege of listening, here, to what marked one of the high spots in after-dinner speeches before this august society, especially from the standpoint of entertainment. If I quote the opening sentences you will recognize the speaker to whom I refer, and I think you will also readily see why I am disconcerted. He began like this:

"Our Society requires its retiring president to close the annual meeting with a discourse or sermon—a task which has become increasingly difficult, for every year the program of the morning and afternoon sessions becomes more abstruse and therefore makes greater demands on our attention and the lingering memories of past presidential rhetoric invite to more odious comparisons. To me the task was the more arduous, because I

[1] Address of the retiring president of the American Society of Naturalists, Boston, Massachusetts, December 30, 1946. The address was illustrated with lantern slide figures, only a few of which are included here. The original work reported in the last part of the paper was supported in part by the American Philosophical Society and the Rockefeller Foundation.

had been busy for many years in remote fields of entomology in which few of you are interested, and because it fell to me at an inopportune moment, while I was in the very act of laying—if you will pardon a French expression—a volume of some 1,100 pages on ants. This racking oviposition leaves me reduced to a mere blob of corpora lutea and so feeble that I can only crawl, in search of a text for my sermon, to the next Encyclopaedia Britannica article, which is not 'ant-eater,' but 'Antaeus.' ''

The speaker, of course, was Harvard's William Morton Wheeler, here on his own home ground. His topic was "The Dry-rot of Our Academic Biology"[2]—a subject which delighted his soul, and which in his hands delighted his audience even more. At least it delighted all those who, like myself, were around the tender age of thirty and hence could get huge enjoyment out of the squirms of our sedate elders as Wheeler's shafts went home. Then, as now, our society was supposed to be very dignified. The speaker's table was large and was on an elevated platform, so many of the elder statesmen—recipients of Wheeler's darts—flanked him on either side (like the gentlemen about me) in full view of the rest of us. I would like nothing better than to entertain you to-night with Wheeler's address. But custom forbids second-hand orations. A failure to cite any of his remarks, however, would be an equal injustice, particularly in view of the fact that we would thereby miss the opportunity to compare the pathological symptoms of a quarter of a century ago with those of to-day and also to glean some bits of philosophy which are pertinent to this evening's discussion. So let me recite a few short passages, mainly from the early part of his discourse.

Where we left off Wheeler was speaking of Antaeus. He went on to recount the exploits of Antaeus, who, as you know, was invincible as long as he was in contact with the earth. But "one day Hercules came along and, knowing the secret of the giant's strength, raised him aloft

[2] W. M. Wheeler, 1923. *Science*, 57: 61–71.

and strangled him in the air." Wheeler used this as an object lesson for biologists, saying that the "meaning of the myth is that even an agile and vigorous mortal had best keep his feet on the concrete if he wishes to avoid death at the hands of the Hercules of abstraction" and that "the myth remains to this day as one of the most beautiful expressions of the practical man's attitude toward those who place too much confidence in their more abstract intellectual operations."

From this he turns more specifically to biology professors. "After securing this text (he says) there was difficulty with the title of my sermon. I could not decide whether to call it the 'tommy-rot' or the 'dry-rot' of our academic biology. I finally chose the latter, because some of our activities so closely resemble the inroads of the fungus *Merulius lacrymans* in old timber. . . ." Then he referred to "the disappointing spectacle of our accomplishments as more or less decayed campus biologists" and to his opinion that "even if we concede that the damned professor (here he is quoting Bismarck) is an extraordinary being because he has sufficient inertia to specialize for a lifetime in a particular department of learning, we must admit that he will grow old like the most ordinary individual of his species. . . . At forty, if not sooner, his sense-organs, musculature, endocrines, emotions and memory will begin to atrophy and his intellectual processes will become more and more stereotyped, dogmatic and abstract. . . . he will become a creature increasingly infatuated with generalizations, relationships and hypothetical explanations, . . .

"Unfortunately we have no intelligence tests for individuals with a mentality of more than eighteen years, and biologists are supposed to be older, though some of them somehow manage to harmonize a physical age of forty to sixty with a mentality of eight to fourteen." However, "it is not from such professors that the Merulius spores proliferate most profusely, but from those who have a physical age of forty to sixty and a mental age of eighty to one hundred and five."

Perhaps these samples will serve to give you a portrait of the biology professor of Wheeler's day. You must be the judge of whether or not he has changed in the past quarter century.

And now, in turning to what is supposed to be my real topic, let me pick out just one more bouquet from Wheeler. This one could hardly have been improved if it had been aimed directly at us to-night. Speaking of the esoteric efforts of professors he says: "This type of senescent compensation is . . . clearly exhibited by old or prematurely old taxonomists, morphologists and geneticists, who derive from static fictions like species, unit characters, genes, etc., a certain feeling of potency, of having their fingers on the very vitals of organic reality."

With this last remark ringing in our ears should any one of us have the temerity to attack such a problem as I have chosen for this evening's discussion? Probably not. Probably by so doing, I admit membership in that numerous company of "more or less decayed campus biologists." But then, I am past forty and presumably beyond hope, so please bear with me. At least these quotations from Wheeler ought to convince you that I realize the nebulous nature of the ideas I may deal with when I get aloft and well out of contact with mother earth.

As has often been pointed out, evolution can not be explained on the basis of loss or simple alteration of materials already present in the germ-plasm. New elements must be added. Otherwise, we would have to assume that the "primordial amoeba" was endowed with all the germinal components now present throughout the wide range of its descendants, from protozoa to man. It is this problem of acquiring new germinal materials which concerns us. Observing Wheeler's admonition to keep our feet on the ground, at least for a few moments, let me begin by directing attention to some of the simpler and more obvious aspects of the subject.

The discussion will be confined to the chromosomes. Whether or not we agree that the chromosomes represent

the germ-plasm, we may be confident that they represent a major part of it—a part more than ample for present purposes. Our primary task, therefore, is to get some conception of how new materials have been added to the chromosomes during the course of evolution.

To enter effectively into the complex genic organization of a chromosome, a new unit would first have to be of the proper size; it would also have to be self-reproducing; it would have to be able to correlate its rate of growth and reproduction with those of the other genic units; it would presumably have to possess the peculiar ability to fit into the so-called "gene-string," attaching itself in the proper manner to its neighbor units on either side; and it would, of course, have to be able to function in harmony with its compatriots. Conceivably, the so-called "plasmagenes" now coming to light, especially in the studies on microorganisms, may prove to be such units. But at the moment it seems more probable that they have been derived from the nucleus than that they are potential contributors to the nuclear mechanism.

From the practical standpoint, therefore, we need only consider additions to the germinal material which come primarily from the chromosomes themselves. If we add to the chromosome complement of a nucleus whole chromosomes, or parts of chromosomes, we are adding something which already has the properties necessary for perpetuating itself.

Addition alone is not sufficient for evolutionary purposes, however, as has often been pointed out. We have not added anything new. Qualitative changes must ensue in the duplicated materials. We can not assume that present-day animals and plants have evolved from the "primordial amoeba" merely through the process of multiplying the relatively simple germinal constituents originally present in that primitive organism.

Were there time, it would be interesting to speculate on how much chemical resemblance there may be between to-day's genic "units" and the primordial ones. Suffice

it to say that to-day's genes, or many of them, are probably much more complex than their remote ancestors. Considering the variety of characteristics presumably common to all genes as we know them, it is not unreasonable to suppose that to-day's genes have all descended from one original gene. We may picture that gene as multiplying to give many replicas of itself. Subsequently, some of these underwent qualitative change, while others remained as they were and continued to perform their old functions. This process continued. Sooner or later chromosomes were formed and groups of germinal materials acted as units. Once embodied in chromosomes, the genes had to act in concert, laying the foundation for the present-day precision of chromosome behavior. For continued evolution, further additions of germinal material were required from time to time. Here duplication of chromosome parts came in as a major factor. If units A, B, C became duplicated in a chromosome, any one of them could undergo qualitative change while its counterpart kept on performing its old functions. With subsequent modifications and adjustments in function of other genes, the unchanged counterpart might itself become modified or lost without detrimental effects. Thus, ultimately, the original, simple germinal materials might no longer exist as such in the genetic mechanism. Presumably, repeated additions and qualitative modifications could transform all the germinal material in the course of time and give rise to any degree of complexity compatible with the proper functioning of the cell and the organism in the struggle for survival in nature.

In the latter part of this excursion our feet have admittedly soared well off the ground. But perhaps the flight has served to emphasize the two major aspects of our problem, first, the addition of chromosome materials, and second, their subsequent qualitative change. We are primarily interested in concrete evidence and in interpretations to be derived therefrom, but unfortunately, the

subject is one about which we know relatively little; hence the great temptation to speculate. My main reason for treating the topic at this time is its importance. There are additional reasons, however; one is the fact that a large part of our specific information in the field in question has been obtained within the past ten or twelve years and is not yet widely known. Another is that it is interesting at this stage to see what paths may be open for further progress.

Needless to say, it is impossible to give an adequate review of our subject in a discussion like the present one. We must treat mainly not clear-cut facts and demonstrated results, but preliminary evidence, indications, probabilities and possibilities, most of which would need full discussion for an adequate understanding. So, for the most part, I will have to confine myself to general aspects and interpretations, without attempting to elaborate experimental details. And I will have to omit all discussion of methods and mechanisms—such as the mechanisms by means of which chromosome rearrangements are brought about.

Simple doubling of the chromosome groups to produce tetraploidy would seem to be the ideal first step toward our goal of increasing the chromosome materials, and this process appears indeed to have performed an evolutionary function in some groups of plants. Nevertheless, the evidence seems clearly to show that polyploidy is not a prime factor in evolution generally, particularly among animals. Likewise, there are good observational grounds for ruling out the addition of single, whole chromosomes as a broad basic evolutionary process (presumably because of the deleterious effect of the unbalance thus created). This leaves us, then, with the particular subject of to-night's discussion: "The Duplication of Chromosome Parts." To my mind, the duplication of chromosome parts, together with its consequences, has probably been one of the most important factors in evolution, if not the most important. But we must admit that

the inference is arrived at by a process of elimination rather than from specific evidence.

Up to 1933 progress was very slow in the field in question. Comparative cytological studies on chromosomes of different species, and genetic evidence of translocation of chromosome parts (*e.g.*, that of Bridges in 1918) suggested that chromosomes are occasionally enlarged by the addition of pieces without compensating loss. But these studies furnished little precise information about what happened or how it happened. A similar suggestion came from the genetic evidence of so-called "duplicate genes"—that is, genes which appear to be duplicates of one another in their genetic effect, but are located in different chromosomes or different parts of one chromosome. Such cases are known in both animals and plants —and, of course, suggest an origin from duplication of chromosome parts.

An especially significant step along this line was made when Sturtevant showed in 1925 that the dominant mutant Bar-eye in Drosophila arose through duplication of something already present in the chromosome. Bar arose as a spontaneous mutation. Subsequently, other comparable cases have come to light in various organisms, especially among the mutations induced by irradiation.

It was not until the giant salivary gland chromosomes of the Diptera came into the picture in 1933, however, that the field became wide open for an accurate determination of what was taking place in such cases. Our fondest dreams could hardly create anything more nearly ideal for the purpose than these enormous chromosomes found in the larvae of Diptera. For any of you who may possibly be unfamiliar with them, let me indicate some of their peculiar advantages. Their size and general characteristics in comparison with their counterparts in "ordinary" cells are shown in the photomicrograph (Fig. 1). This figure is from one of the fungus flies, Sciara. The enormous size of these chromosomes

may be seen by comparing with the chromosome figure in the lower right-hand corner, which is a photomicrograph of the chromosome group in an ordinary mitotic cell of this same species taken at the same magnification. As you will see, the species has four pairs of chromosomes and each salivary gland chromosome represents a pair. In other words, what appears to be a single salivary gland chromosome is really a pair of homologs intimately

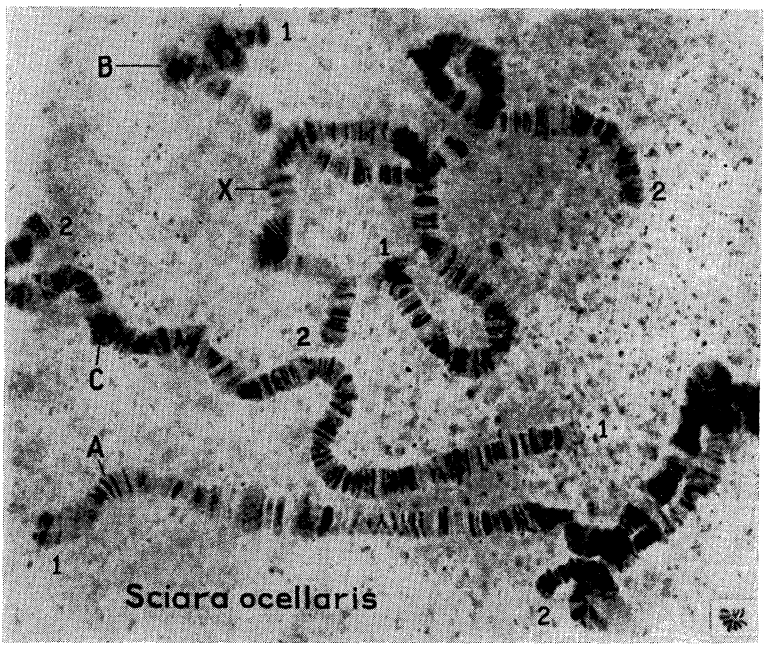

FIG. 1. Photomicrograph of salivary gland chromosome group of the fungus fly *Sciara ocellaris* Comstock, from an aceto-carmine smear preparation. ×575. See text for explanation.

united side by side so that each chromatic cross-band or disk in the one is joined to its mate in the other—the two together making a continuous band or disk across the more or less cylindrical "chromosome." These cross-bands or disks (they are actually disk-shaped) have individual characteristics of thickness, degree of staining, etc., which differentiate them from one another, with the result that not only each chromosome, but even any short

region within a chromosome, has its own distinctive pattern which distinguishes it from other regions. This pattern is highly constant and makes possible an accurate comparison of chromosome regions.

Needless to say, the Bar-eye mutant in Drosophila was one of the first subjects to be investigated with the aid of the giant chromosomes. The Bar flies were found by Bridges, and independently by Muller, Prokofyeva-Belgovskaya and Kossikov, to possess a duplicated piece of the normal chromosome, consisting of a segment contain-

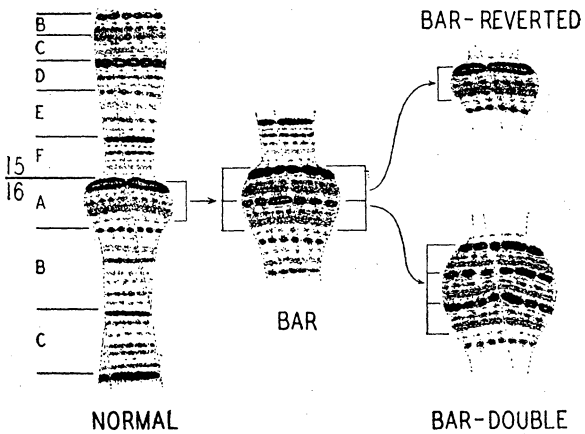

FIG. 2. The Bar duplication in *Drosophila melanogaster* as seen in the salivary gland chromosomes. (After Bridges, 1936, *Science, 83:* 210–211).

ing at least seven of the chromatic disks or cross bands. The condition in the normal chromosome is shown at the left in Fig. 2. The Bar segment is enclosed in the bracket. The condition in Bar is shown in the middle, where this segment is duplicated. These investigations also corroborated Sturtevant's earlier evidence that "Double-Bar" and other extreme forms were the result of further duplications of this same segment. The process of unequal crossing over which gives the triple condition from Bar may also cause a reversion back to the single condition, as shown at the right in Fig. 2. Another significant aspect, as shown by Sturtevant, is that the effect of the duplicated segments depends not only on

their number but on their spatial relations. In other words, we have here not only a duplication of parts, but what is known as a "position effect." For example, if three Bar segments are present in one homolog of the pair, and one is present in the other, the phenotypic effect is more extreme than that produced when two Bar segments are present in each homolog.

Studies on the salivary gland chromosomes also soon brought to light another line of evidence—this time based on normally occurring conditions in wild stocks. In an intensive study of the salivary gland chromosomes of *Drosophila melanogaster,* Bridges found cases of duplicated or "repeat" regions which are characteristic of the normal chromosomes of the species. Such regions are not only alike in their band patterns but tend to show their homology by uniting with one another side by side in somatic synapsis.

Some of these repeats are essentially similar to the Bar repeat except that the pattern in one segment is reversed and thus is the mirror image of that in the other segment. Such a repeat is an adjacent, reversed repeat, whereas Bar is an adjacent, serial repeat. Another type of repeat differs from these two in that the repeat segments are not adjacent but well separated. In such cases the repeat regions usually come together in somatic synapsis and when forcibly separated during preparation of the "smears," lateral strands of chromosome material connect homologous bands in the two segments (see, *e.g.,* Fig. 5, regions 33–34 and 38–39, Bridges, 1935, *Jour. Hered.,* vol. 26, p. 62; also Fig. 4 of present paper).

All the repeats considered up to this point consist of several bands, involving in each case a definite pattern. For convenience such repeats may be called "pattern repeats." Bridges immediately pointed out the possible evolutionary significance of these repeats. In the fungus fly Sciara we also found such conditions, to which I will refer again presently. Adjacent repeats are apparently more numerous than well-separated ones in wild popula-

tions—and for reasons that will appear later they are, so far as known, always "reversed repeats."

In addition to these "pattern repeats," which unquestionably represent duplications, there is another type of structure found commonly in salivary gland chromosomes which may also be derived by duplication. This is the so-called "doublet" originally identified by Bridges and interpreted as a repeat. A doublet involves only two bands—one band supposedly the duplicate of the other. An example of a doublet is shown at the upper left in Fig 3*a* (indicated by the dotted line). The particular reason for supposing the two bands to be duplicates is that they converge at the margin to form a lens-shaped structure, like two saucers placed face to face. The convergence is interpreted as evidence of synaptic attraction, which indicates homology. Our evidence from Sciara tends to corroborate Bridges' contention, as do also subsequent experimental studies on Drosophila. Some authors, however, interpret the doublets as single units.

This matter is important and merits more careful examination than we can give it here. The present state of the subject may perhaps be summarized as follows: Bridges has recorded at least three cases of irradiation experiments which he interprets as involving breakage of the chromosome between the two bands of a doublet, with consequent separation of the two—indicating that the doublet is really a double structure. We have found in wild populations of Sciara cases in which what appears to be a doublet in one chromosome of a pair is matched by a single band in the other homolog. An example is shown in Fig. 3. As you will see, the single band in one homolog resembles half of the doublet. All three possible conditions are found—that in which both homologs have the doublet structure and that in which both have the single band, as well as the heterozygous condition just referred to. Two conditions are shown in Fig. 3. In the chromosome at the left, at the locus indicated by the

dotted line, the doublet structure is present in both homologs. In the other chromosome the heterozygous condition is present. The interpretation of this figure is shown in the diagram. Both the appearance and behavior here suggest strongly that the doublet is the result of duplication.

Several genetic investigations in Drosophila point in the same direction. I will cite only Lewis's study of the

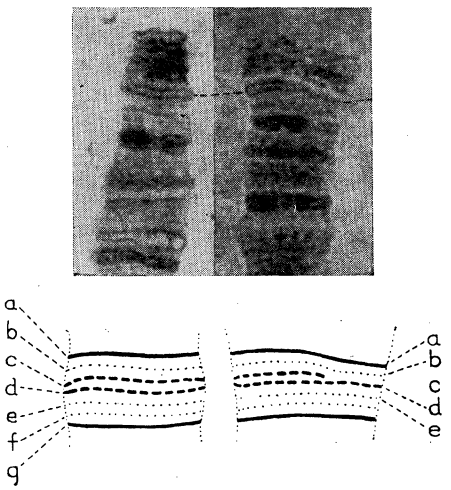

FIG. 3. *a*, photomicrograph, and *b*, explanatory diagram, illustrating the "doublet" condition in the salivary gland chromosomes of *Sciara ocellaris*. Chromosome at left shows homozygous doublet condition at dotted line. Chromosome at right shows heterozygous condition as indicated schematically in the diagram at the right. See text for description. Photomicrograph ×1500.

Star-asteroid locus in *Drosophila melanogaster* (a locus involving a conspicuous doublet). Lewis finds strong genetic support for the view that in this doublet the gene for Star is in one band of the doublet and that for asteroid (a similar but not allelic mutant) is in the other. He did not succeed, however, in separating experimentally the two bands of the doublet.

Such lines of evidence, taken together, make it probable that a doublet is really a double structure, although there are some grounds for questioning the interpreta-

tion on the basis of cytological complications which can not be gone into here.

Whether the two bands of a doublet are really homologous or not, however, is less clear. Lewis's genetic evidence tends to indicate that they are, but needs to be supported by studies on other cases. We are still far from an understanding of the finer details of organization of the salivary gland chromosomes as seen under the microscope, and such studies, like the genetic investigations just suggested, might contribute greatly to a solution of the present problem. In such studies particular emphasis should be given to any possibility of identifying *qualitative* differences between the two bands of a doublet—either cytologically or genetically. If doublets are single band repeats we need evidence of incipient evolutionary change bringing about a difference between the two loci. Needless to say, such evidence will be very difficult to secure or interpret.

Almost the same problems as those just considered are presented by the pairs of bands which do not show the doublet configuration in the salivary gland chromosomes. Are these pairs simply chance associations of similar bands, or do they represent doublets in which the synaptic attraction has disappeared because of qualitative divergence of the two loci? Rapoport expresses the latter view, based on experimental studies on the Bar duplication, in which he secured multiple duplication of the Bar region up to sextuple and octuple. He goes even further than the pairs of bands and makes the proposal that chromosome regions in which several similar bands lie in sequence represent multiple duplications of one original band. Correlating the additive genetic effects with the chromosome changes in the Bar series, he develops the interesting hypothesis that multiple repeats offer one possible explanation for orthogenesis—particularly cases of orthogenesis which paleontologists have regarded as involving especially rapid evolution.

In this connection, reference should be made to the

subject of heterochromatin—that ill-defined, yet bulky, chromosome material apparently present to some extent in all chromosomes. As you know, it is associated with the so-called inert chromosome regions, which appear to be almost devoid of genes and which are relatively more prominent in the mitotic cells than in the salivary glands. The behavior of this material suggests that it may have been derived through large-scale duplication of one or a few ancestral units, and that it may possibly be simpler or more primitive than the so-called "euchromatin."

Another line of evidence, pertinent at this point, comes from several studies on maize, especially those of McClintock and Stadler. I can only cite one—an investigation by McClintock which involves both cytological and genetic analysis. McClintock was able to identify two very short, adjacent segments near the tip of chromosome 9 in maize. Loss of the distal one resulted in a chlorophyll deficiency giving pale-yellow seedlings. Modification (or, presumably, loss) of the other likewise caused a chlorophyll deficiency—giving yellow-green seedlings. Simultaneous loss of both segments gave complete chlorophyll deficiency—white seedlings.

The significant aspect of this situation is the fact that we have adjacent chromosome regions influencing in an additive manner the production of a quantitative character—chlorophyll intensity. There are other similarities in their action which I have not included here. On the basis of her results, published in a series of papers, McClintock has developed an unpublished interpretation which she kindly permits me to cite here. On this interpretation, the regions under discussion near the tip of chromosome 9 are regarded as made up of a series of similar or identical genes, each of which contributes a specific quantity of substance necessary for chlorophyll production. They therefore have an additive effect. Such a series would be interpreted as having arisen through duplication of individual loci or short chromosome segments. The evolutionary possibilities in this

case are evident—and, of course, one is tempted to apply them to the whole problem of the origin and evolution of chlorophyll production in plants.

If we wanted to turn back to Drosophila we could likewise find evidence from various genetic studies supporting the thesis that adjacent chromosome regions or loci often have similar genes and may represent duplications, as Schultz has pointed out. The Star-asteroid case investigated by Lewis is cited only as a particularly clear-cut example.

Obviously, studies such as those just cited are only suggestive; but they open up a large field for investigation and speculation. Suppose we assume that the series of similar single bands found here and there in the salivary gland chromosomes do represent multiple repeats—then what about other bands in the chromosomes which resemble these particular ones? Are they homologs also? They could readily have been separated from the series through inversions of larger segments of the chromosomes. From this it is only a step to the grouping of all the bands of a chromosome into a few classes on the basis of their morphological similarity and implying that all those in each class are homologous—with the result that we would have only a few kinds of genes and have many representatives of each kind, with minor grades of difference within the classes. Thus we could reach almost any height of speculation.

So much for single band repeats—possible or real. It is something of a relief to turn from them back to the category of duplications we began with—the ones we know are duplications. These exhibit clear-cut band patterns which can be matched, and in favorable cases they exhibit the lateral synaptic attachment, band for band, which is regarded as the final evidence of homology.

Before considering other actual cases of duplications in detail it may be useful to look at some of the general characteristics and potentialities of different kinds of duplications. In the first place, of course, to be of evolu-

tionary importance a duplication must be such as to permit survival of the cell and the organism. Consequently, we would expect repeats that are established in natural populations to be reasonably small, at least in higher organisms, because otherwise they would be apt to upset the genic balance. Evidence from the salivary gland chromosomes tends to fulfill this expectation. Secondly, if the duplication is to persist and function in evolution, it presumably must have or soon acquire a beneficial function—for example, in ordinary plants an increase in chlorophyll production, such as that detected by McClintock in maize. In the third place the duplication must be such as to become stabilized in the population or species. Some types of duplications have the latter characteristic; others do not. This may be illustrated by referring again to the Bar case. The Bar duplication is inherently unstable and shows its instability, as already noted, by giving rise to higher multiples which in turn may revert back to lower ones and even to the original wild-type condition. The reason for this is that the repeated regions are adjacent to one another and are in series—*i.e.,* are oriented alike—not reversed in sequence. Consequently, unequal crossing-over can readily bring about the changes just noted. In natural populations this kind of duplication is conspicuous by its absence. Instead of it we find the so-called "reversed repeats" when the repeated regions are adjacent.

As other authors have pointed out, the stability of reversed repeats is due to the fact that unequal crossing-over here leads to non-viable chromosome conditions—*i.e.,* modifications that do not persist—which is not necessarily true in the serial repeats like Bar.

This situation presents interesting possibilities. The characteristic is both beneficial and detrimental. It tends to insure the required stability of chromosome organization; but at the same time it reduces the number of viable germ cells produced in proportion to the amount of crossing-over. This raises the question as to how many re-

peats and how large ones an organism can tolerate without damage from this non-viability. I am sorry time does not permit exploring this question further.

Another question raised here concerns the matter of single band repeats. If serial repeats are inherently unstable how could a series of two or more single bands, such as doublets, pairs or higher multiples, become stabilized? We might assume that when only two bands are present one is reversed (as suggested by Lewis) and that this gives stability; but so far as I know we have no evidence that such reversal would have this effect here, and in any event it would help us little where four or more bands are involved.

Further interesting information about repeats comes to light when we compare conditions in different organisms, and when we examine certain individual cases. Take, for example, the condition found frequently in wild populations of Sciara, in which one band of what appears to be a doublet in one chromosome of a pair is lacking in the other member. This condition is found frequently in wild populations of Sciara, but seems to be exceedingly rare in Drosophila. Should we infer that evolutionary change in this respect is going on relatively rapidly in Sciara? Unfortunately, no obvious external modifications of the flies accompany these conditions, so we have no evidence as to whether any one condition is more valuable to the organism than the others.

In the case of the larger duplications (as well as the doublets, etc.,) there is evidence in both Drosophila and Sciara that in very closely related species conditions are similar, especially in cases where hybrids have been secured. But exact information on this point is relatively meager thus far, because of technical difficulties which I will come to in a moment. In Drosophila, according to Dobzhansky, there is a tendency for repeats, when found, to be concentrated in particular chromosomes. And even when species are compared that are not closely enough related to be hybridized, these par-

ticular chromosomes are found to be ones which, on other grounds, are considered to be homologous in the different species—which suggests common origin and evolutionary relationship. In Sciara, a similar indication is seen in the repeats found in the X chromosome of several species. This situation is under investigation at the present time, and one aspect of it warrants special notice here, even though the study is far from complete.

In at least four species of Sciara the X chromosome exhibits a new type of repeat condition—one in which the repeated region appears to be represented three times in different parts of the chromosome. Two of these species, *S. ocellaris* and *S. reynoldsi,* are very closely related and will hybridize. In them, the "triple" repeats have been studied intensively. The technical difficulty of getting good figures is greater than ever here where there are two sets of synaptic attachments. The stresses resulting from these attachments when the "smears" are made, almost invariably distort the pattern in the repeat regions. Hence, unfortunately, some points are not entirely clear as yet; but the general aspects seem to be evident. The triple repeat conditions in the two species seem to be identical and the following description applies to both. As the photomicrographs (Figs. 1 and 4) show, the X chromosome is typically in the form of a figure 8 in which both ends of the chromosome are folded back. A short region near end-1 is attached laterally to the two other parts of the chromosome which lie very close to it in the figure, thus holding the chromosome in this configuration. The attachments are shown in Fig. 4. Some of the bands near end-1 are continuous at the left with bands not far from end-2 and the same bands extend out to the right connecting with bands in the third repeat region. Further details which do not show in the photomicrograph appear to be as follows. The repeated segment involves four bands, no two of which are exactly alike. Each of the three segments has the same pattern. Synapsis apparently occurs between all four bands in one segment and the corresponding ones in another.

For convenience I have designated the segment near end-1 of the chromosome (shown in the middle) as R-1 and the other two as R-2 and R-3 respectively.

At first it was assumed that the synaptic relationship between the three repeat regions here would be alike.

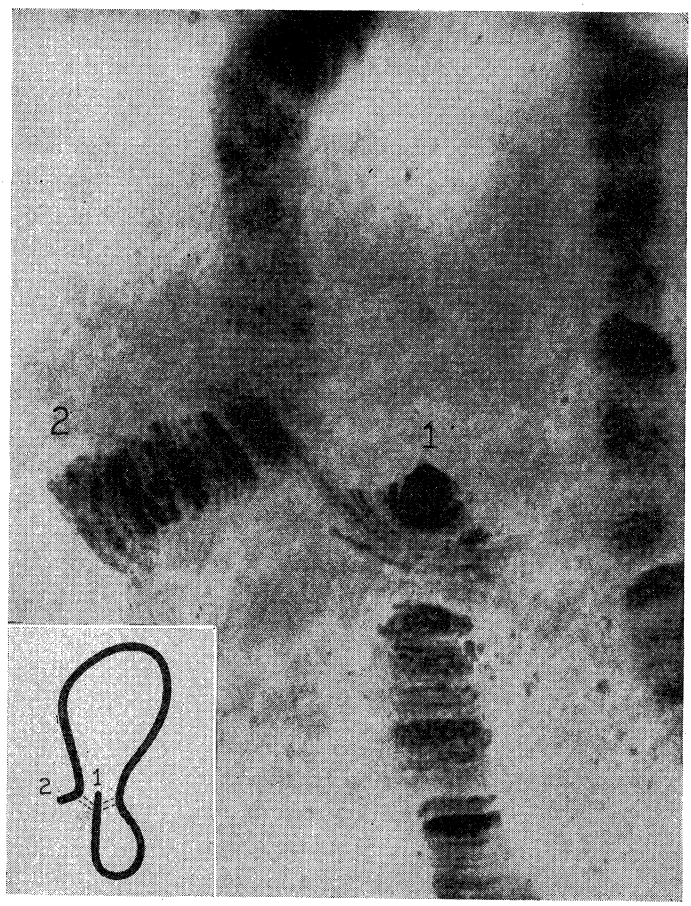

FIG. 4. Photomicrograph and explanatory diagram showing the "triple" repeat condition in the X chromosome of *Sciara ocellaris* Comstock. See also Fig. 1. Explanation in text. ×1500.

But not so. Examination of hundreds of figures has shown that apparently without exception synapsis occurs only between R-1 and R-2, and between R-1 and R-3, but not between R-2 and R-3. This is true in both species.

Such selective synapsis seems extraordinary, for we know from evidence from triploid conditions in Drosophila and elsewhere that in the salivary glands three homologous chromosomes can associate uniformly and intimately. Even if we assumed that for some reason synaptic attraction in the Sciara case were satisfied by a two-by-two association we would expect random union between the three repeat regions. Regions 2 and 3 should unite as frequently as either does with region 1. Unless our observations are at fault, therefore (a possibility which can not be ruled out at the moment), we can only conclude that there are different degrees of homology here in spite of what appear to be morphologically similar patterns. On a qualitative basis R-1 and R-2 have something in common which results in synapsis; similarly, R-1 and R-3 have something in common, but a different something from the other; but R-2 and R-3 do not have either quality in common. Are we to conclude from this that R-1 represents an ancestral condition from which R-2 and R-3 have evolved qualitatively in different directions? At the moment this seems to be one of the possible interpretations. In any event, the present condition presumably came into existence before the two species became distinct from one another. In other words, not only was the triple repeat present in the ancestor of the two species, as we have suggested in earlier papers, but the qualitative or other changes must have taken place in that species or in its ancestors, unless we make the improbable assumption of independent origin.

Not enough is known as yet about the triple repeats in the other two Sciara species just mentioned to warrant more than the statement that the size and the location of the repeat regions are such as to suggest the possibility of a common origin with the regions just considered.

A second feature of the triple repeat case is what may be called the unit action or behavior of the repeat region. In the triple repeat, why should the three regions be the same size? In other words, why should each have re-

mained complete and intact over such a long period of time? It looks as if the region behaves as a unit either in the sense that it can not be fractionated, which seems improbable, or that it performs a unit function for which all parts are essential. The synaptic behavior points in the same direction. Is there a primary locus or gene in the segment which acts as a controlling agent, but which requires the presence of the others for proper functioning? And are we dealing with a situation involving position effect, in which the parts must all be in proper relationship to one another? Much could be explained on that basis, not only in the case of triple repeats, but in ordinary repeats. It looks very much as if this unit action may be characteristic of all established "pattern repeats"—*i.e.*, repeats involving more than one kind of band. If these surmises are correct, we may wonder how many other regions in the chromosomes also represent units of action in this same sense.

Such comparisons could be continued at considerable length; but I fear I have already tried to cover too much ground.

And now to recaptiulate and bring together what has been presented: We may distinguish, I think, three types of conditions which have particular interest for us. One type may represent duplications of single loci or disks; this includes the doublets, the pairs of disks and the series of three or more similar disks. If these all represent recent duplications, then apparently all the well-known salivary gland chromosomes are full of duplications—which, on our present view, would not be surprising. Two major uncertainties face us here, however. First, do these conditions really represent duplications? Second, if they do, how did they get stabilized? Why does not unequal crossing over cause great variability? The other two types of conditions represent duplications of larger chromosome regions—they are the "pattern repeats"—the adjacent repeats and the non-adjacent repeats, respectively. The distinction made between them

is arbitrary, but useful. In adjacent repeats a reversed orientation is ordinarily found—presumably required for stability. It is difficult to see how a series of more than two such regions could become established except under special conditions (such as absence of crossing over in the repeated regions). In the non-adjacent repeats, however, orientation appears to be unimportant, and more than two repeated segments may be present (as in the triple repeats in Sciara). Like the single band repeats, this type should be capable of indefinite multiplication; but unlike the single-band repeats these should be capable of attaining stability without interference by unequal crossing over (except under special conditions).

If this résumé is correct, then, we would expect the three kinds of duplications to have different evolutionary potentialities, as just indicated.

Such is the picture, at least as I see it. If I were not past forty, and if I did not have to worry about the inroads of *Merulius lacrymans,* I would present a peroration at this point. As it is, however, I think I had best be content with expressing an opinion. For the most part, the discussion this evening has served to raise questions without giving the answers. I realize that many of the answers are shrouded in uncertainty. I realize also that we have scarcely more than crossed the threshold of analysis of the main problems. Nevertheless, I think much progress has been made in the past ten or twelve years. And I think we now have available both the material and the methods for making much greater progress in the future. So I think the field presents a stimulating challenge to any one who would help unravel the secrets of evolution (especially if he is under thirty).