VARIEGATION IN DROSOPHILA AND THE INERT CHROMOSOME REGIONS

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At the time of their discovery, the variegated races of Drosophila (D. virilis, Demerec ('26); D. melanogaster, Muller ('30)) seemed to be promising material for the study of mutation. They have since developed rather into a puzzle *sui generis*. Members of the group have been discussed variously as mutable genes (Demerec ('28)), unstable translocations (Muller ('30), Patterson and Painter ('31, '32)) and, more recently, as a rather special type of duplication undergoing frequent somatic crossing-over (Stern ('35)). The discussions have not been very satisfactory; partly because the data themselves have been inadequate for the formulation of general rules, excepting only Muller's correlation of the "eversporting" types in D. melanogaster with the occurrence of chromosome rearrangement.

This paper summarizes the results of experiments with thirteen different variegations in Drosophila melanogaster. All of these belong to Muller's "eversporting displacements"—they are associated with chromosome rearrangements. Five involve the region around the white locus in chromosome 1; two the yellow region of the same chromosome; and the six remaining, the brown region of the second chromosome. I have attempted to determine what relation there might be between the nature of the rearrangements and the production of variegation. To this end, I have studied the characteristics of the rearrangements cytologically, in the salivary gland chromosomes; and the characteristics of the variegations, genetically, in relation to the different affected genes. Both series of data show a relation between variegation and the so-called "inert" regions. The first evidence of the sort came from the work of Gowen and Gay ('34), on the suppression of white-variegation by a supernumerary *Y* chromosome. This may now be extended; extent of variegation, that is, the proportion of "mutant" to wild type tissue, depends upon a quantitative relation between active and "inert" chromosome regions. In addition, however, the "inert" regions are involved in the rearrangements themselves. Thus both the chromosome structure associated with variegation and the extent of variegation are related to the "inert" chromosome regions.

The variegated races form abnormal configurations of the salivary gland chromosomes as a direct result of their relation to the "inert" regions. In a nucleus with normal chromosomes, the "inert" regions are aggregated to

form a "chromocenter" (Heitz ('33, a, b,); Painter ('35)). Since in Drosophila melanogaster these regions are normally located near the spindle attachments of the chromosomes (Heitz ('33), Kaufman ('34)), a configuration is formed in which all spindle attachments are approximated. In the chromosome rearrangements where "inert" regions are transferred from the spindle attachment to other loci, the coalescence to a chromocenter persists in the salivary gland nucleus. The result is that, in nuclei of such types, rings, lateral attachments and more complex configurations may occur.

Ring configurations are regularly found when an "active" chromosome region is intercalated into an "inert" region. For example, both ends of the normal fourth chromosomes of Drosophila melanogaster are regularly part of the chromocenter (Bridges ('35)). Three of the variegations involving the white locus turn out to be intercalations, into the fourth chromosome, of the section of the *X* from yellow to white, or in one case, from yellow to echinus. These form, then, part of a ring configuration. A similar situation exists for the yellow-silver variegation of Sturtevant ('34), scute 10-2. Another variegated white forms a ring as the result of intercalation of a small portion of the *X* chromosome, including white, into the inert region of the left limb of chromosome 3.

Effective "lateral" attachments occur when "inert" region is intercalated between two "active" regions. After chromocenter formation, the "active" region distal to the inert material is attached to the chromocenter by its proximal end; the other "active" region, between the inert material and the spindle attachment, is bent into a loop. Figures of this kind are found in the scute-8 inversion, in two of the variegated brown allelomorphs, and in one of the white variegations. It is readily seen that the principle is the same as in the cases of ring formation. Still more complex configurations are found in other cases, the four remaining variegated brown allelomorphs. They also result from the breakage and rearrangement of inert regions, which by their aggregation to form a chromocenter may then join the broken chromosomes. An apparently similar case has been described by Dubinin and his collaborators ('35), and interpreted as a fusion of specific intergenic bonds. Evidently on the basis of the present discussion this assumption is not yet necessary. Their "reconstruction of a normal chromosome" presumably followed disruption of the chromocenter aggregate when the preparation was smeared.

In addition to the thirteen cases I have studied, those already described by other workers lend themselves to a similar interpretation (see Patterson ('32, '33), Gowen and Gay ('34), Glass ('34a, b), Van Atta ('32), Painter ('34), Mackensen ('35), Patterson and Stone ('35), Stone and Thomas ('35)). There are some variegations, that I am studying, which may be of a different type; and I have seen similar abnormal configurations unac-

companied by obvious variegation. These exceptions are, however, in a minority. The evidence seems adequate to establish the rule, that variegations are associated with abnormal configurations of the salivary gland

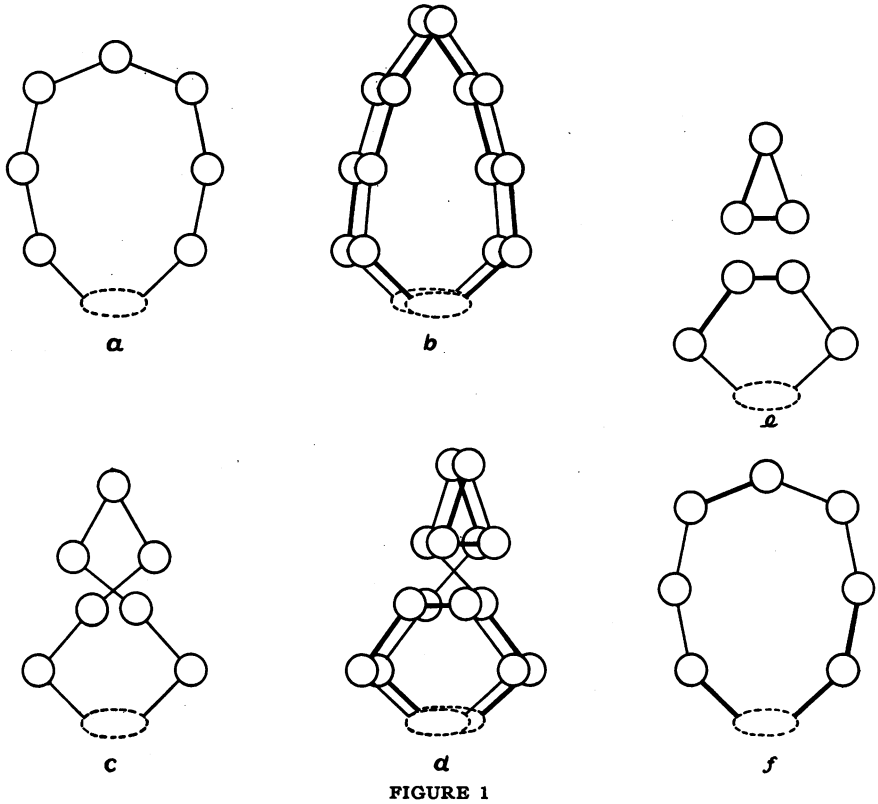

FIGURE 1

Reproduction in normal and twisted ring configurations. The circles represent chromomeres (genes), the ellipses the spindle attachment. Light lines are the old connecting fibres, heavy lines the new ones.

(*a*, *b*) Reproduction in a normal ring; (*c*) a twisted ring; (*d*) reproduction in a ring where distances are such as to permit abnormal linkages to form; (*e*, *f*) the products of reproduction: normal ring, deficient ring and fragment without spindle attachment.

By variation of the type of overlap or the plane of formation of the new fibres, rearrangements, duplications, double or interlocked rings may result.

chromosomes, caused in these cases by the aggregation of inert regions to a chromocenter.

The variegation in all of the rearrangements concerns those genes close to the locus of the break. All the available mutants located in the regions showing abnormal configurations were tested for the display of variegation. The evidence from these tests shows clearly that only those characters

whose genes lie close to the locus of rearrangement as determined in the salivary gland chromosomes are affected. No unequivocal instance has yet been found of the total loss of a fragment as the cause of variegation. One such case has been reported to have been seen cytologically (Patterson and Painter ('31), Patterson ('32)). But the genetic data (Patterson ('32)) indicate variegation only for the white-Notch loci, although tests were made for other genes. If the whole fragment were lost, all the genes that are included in the region should show variegation. It would seem, then, that in this case, as in those studied and recognized as being of this nature by Gowen and Gay, and by myself, only a part of the fragment is involved in the variegation.

Changes in the extent of variegation occur when the normal balance of active and inert regions in the nucleus is upset. The suppression of variegation by an extra *Y* chromosome is not confined to the white-Notch cases studied by Gowen and Gay. A similar phenomenon occurs for the brown variegations (Schultz and Dobzhansky ('34), Dubinin and Heptner ('34)) and for the others as well. I have now determined the effects of changing the number of *Y* chromosomes, on variegation for most of the affected genes in the different stocks. In addition, I have found that not only the inert region from the *Y*, but also its relation to the active regions, is important. The evidence for this comes from the study of many different combinations of variegations, in types with different balance of active and inert region. The different fragments of *X* and *Y* chromosomes have been studied; triploids with different numbers of *Y* chromosomes; intersexes also, and superfemales; and particularly certain duplications for active regions, some of which annul, others of which simulate, the effects of the *Y* chromosome. Here I will discuss the effects on variegation of changing the number of *Y* chromosomes in the diploid.

The variegated brown allelomorph Plum-2 may be taken as an example, since it illustrates most of the relations found also in other cases. Flies containing a Plum-2 second chromosome display, when appropriately tested, four distinct types of variegation: a dominant brown (eye color), a recessive brown (eye color and testis sheath), a "minus" bristle and "light" (eye color). Of these, the first three named lie close to one side of the breakage point; the fourth ("light") is at the other of the two breaks, within the inert region of 2*L*. It will be recalled here, that in Plum-2 homozygotes, all the broken portions of the chromosome are joined at the chromocenter.

The characteristics of these variegations have been studied in all types from the *XO* male to the *XXYY* female. Minus, which is to the right of brown, shows an extent of variegation in the *XO* male comparable to that shown by brown in the *XY* male. The susceptibility to variegation decreases as the number of *Y* chromosomes increases. In the case of the

"light" variegation, an apparently converse relation holds. With increase in the number of *Y* chromosomes, the extent of "light" areas in the eye increases; in the *XO* male, however, the eye is completely wild-type as regards the "light" character. This is to be interpreted as meaning that the product of variegation in this case is wild type, and the original form "light." Support for such an interpretation comes from the study of several other cases involving the "light" locus, in which a similar break in 2*L* has produced a similar "mutation" (position effect) to "light." Thus the variegation in this case is a "reversion"to the wild type. On this basis, the effect of addition of *Y* chromosomes in all cases is to decrease the frequency of variegation.

All the variegations—those for yellow and achaete, for white, split and Notch, and the others for loci in the brown region—exhibit similar characteristics. They cannot be discussed here due to limitations of space. In all cases, however, it appears that the extent of variegation increases as the *Y* chromosome number is lowered. The result is that the apparent direction of "mutation," as judged by relative proportions of mutant and wild type tissue, may appear to change with change in *Y* chromosome number. These relations suggest that the origin of variegation may be considered as the result of some type of unstable configuration which produces different types of "mutation" in the different *Y* chromosome types. The presence of the abnormal chromosome configurations in the salivary gland nuclei permits an attempt to formulate an hypothesis based on consideration of the reproduction of genes in these configurations.

The basic postulate for a theory of the variegations involves the nature of the so-called "mutations." Either they are intra-genic, the orthodox mutation types; or they involve structural changes of the chromosomes. In one of the present cases—scute-8—Beadle and Sturtevant (in the press) have shown that germinal deficiency for the variegating portion of the chromosome may occur. Other data, on different variegation types, may be interpreted similarly. These belong then to the simplest type of structural change—a loss which occurs without a complementary production of duplications. There are, in addition, a number of instances of variegation which are definitely different from known deficiencies for the same loci. Examples are the dominant brown variegations, and certain white variegations. These may be interpreted as due to position effects, effectively mutations in appearance, resulting from local rearrangements produced by a mechanism similar to that which produces the deficiencies.

It has long been obvious that losses or rearrangements of genes might occur as a result of the difficulties of division in abnormally shaped chromosomes (see especially the results of McClintock ('32) on ring-shaped chromosomes in Zea mays). It is possible to consider such a process simply, following Belling's ('33) analysis of gene (chromosome) reproduction into

two components. These are the synthesis of a new gene, and the maintenance of the genes in linear order. According to his view, the mechanism which maintains linear order operates after the genes have reproduced. Thus, whenever gene reproduction occurs in crossed chromatids, the new linear connections are formed between the closest genes—hence, according to Belling, crossing-over. This makes no postulate as to the nature of the new "fibre"; what is discussed is the time of its formation.

The application of such an analysis to the variegations is simple. The reproduction of a ring-shaped chromosome will offer a model for consideration. From a normal ring, two identical daughters result (Figs. 1*a*, *b*). But if the ring is twisted so that two non-homologous portions come to lie one over the other, the reproduction is abnormal. When the new fibres form across the shortest way (Fig. 1*d*), a normal ring, a small ring and a fragment lacking a spindle attachment, result under the conditions shown in the figure. Evidently the formation of rings of different sizes, observed by McClintock in Zea mays, might be explained in this way. Moreover, only rings would ordinarily be produced from rings. In addition, by variations of details in the place of twist, or the plane in which the new intergenic connections are formed, a variety of other new rings are possible. These may be gene rearrangements within the ring, or duplications which would appear as larger rings. Further discussion, and the detailed application of the hypothesis to the various cases, is deferred until the full presentation of the data.

The effect of the *Y* chromosome and other inert regions on the extent of variegation may be derived from this point of view, with an additional consideration. I have found, in comparative studies of larvae having different numbers of *Y* chromosomes, differences in the "turgor" of the salivary gland chromosomes. With the increase in number of *Y* chromosomes, all the others show an increase both in the sharpness of stain and the plumpness of the chromosomes. This result may possibly be related to the development of accessory chromosome materials, in a manner similar to the suggestion of McClintock that the nucleolar center in chromosome 6 of Zea mays, has to do with the development of chromosome matrix. Here also a nucleolar center—that of the *Y* chromosome—may be concerned. It is clear that any factor changing the "turgor" of the chromosomes would have an influence on the frequency of cross contacts of non-homologous genes. This would appear as a change of the sort found in the extent of variegation.

The hypothesis that I have indicated for variegation is to be regarded as a pictorial summary of the facts, cytological and genetic, about the variegations. A variety of other hypotheses are possible; differences in rate of gene division, or of formation of intergenic connections; or a whole variety of intragenic changes may be invoked. None that I have considered are

so simply related to the general body of cytogenetic theory as the one I have discussed, although even it has its difficulties. It is perhaps worth noting, that in its more general aspects, the theory presents the study of variegations as a study of the mechanism whereby the genes are maintained in linear order.

[1] A bibliography will be given with the detailed publication of the data.

[2] The terminology is obviously outworn: "inert" is certainly a misnomer. Heitz's use of "heterochromatin" and "euchromatin" for "inert" and active regions is probably better. For the present, however, I have used the older term because of its familiarity.

SPECIAL REGIONS OF REGULARITY OF FUNCTIONS OF SEVERAL COMPLEX VARIABLES

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Introduction.—In the theory of functions of several complex variables various types of regions occur as convergence regions of series developments of analytic functions.[2] We have investigated the problem of the analytic continuation of a function beyond its associated convergence regions. In this note we present a summary of the results of this investigation. A detailed treatment of the subject will be given in a paper which will appear elsewhere.

Let

$$f(x, y) = \sum_{m, n = 0}^{\infty} a_{mn} x^m y^n \tag{1}$$

be any analytic function of the two complex variables x, y, regular at the origin. We shall discuss the analytic continuation of $f(x, y)$ into three classes of regions.

1. *Diagrams.*—With $f(x, y)$ let us associate the class of *Borel transforms*

$$F(x, y;\ p, q) = \sum_{m, n = 0}^{\infty} \frac{a_{mn} x^m y^n}{(pm + qn)!}, \quad (p, q, \text{positive integers}) \tag{2}$$

Each of these transforms is easily seen to be an entire function. By considering the growth of $F(x, y;\ p, q)$ we define real-valued functions

$$h^*(x, y;\ p, q) = \limsup_{\rho = \infty} \frac{\log \left| F(x\rho^p, y\rho^q;\ p, q) \right|}{\rho}, \tag{3}$$