# SMALL DEFICIENCIES AND THE PROBLEM OF GENETIC UNITS IN THE GIANT CHROMOSOMES*

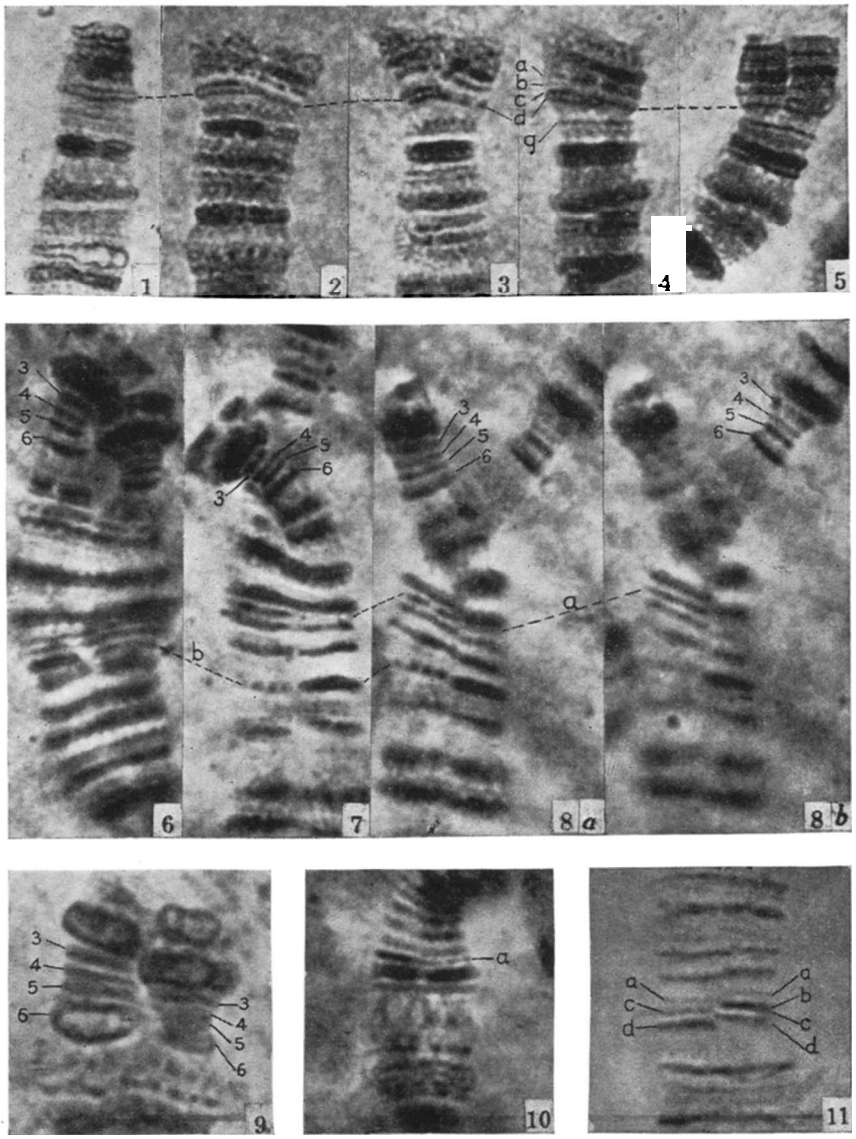
C. W. METZ
*Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland*
Received June 11, 1937

ONE of the main problems involved in studies on the giant gland chromosomes of Diptera is that of visible identification of the genetic units. Two modes of attack on the problem are being followed. On the one hand attention is devoted, through direct observation, to study of structures which look as if they might be units; and on the other, it is devoted, through study of small deficiencies, to structures which behave as units, that is, are acquired or lost as units. The present account deals mainly with the latter aspect, or, more specifically, with two questions which naturally arise in considering this aspect. These are: First, how is it possible to tell when the smallest structure is found which may be acquired or lost as a unit? And second, what is the nature of this structure in terms of the visible chromatic and achromatic constituents in the chromosome?

Since the chromatic material is present in the form of transverse "discs" or "bands" which are readily observed, deficiencies may be identified by detecting "bands" in one homologue which are absent at corresponding loci in the other homologue. One obvious possibility, as suggested by earlier observers, is that a single chromatic band or disc represents a unit locus. But, as all observers have found, the "bands" differ in thickness and many of them are compounds made up of two or more closely approximated discs. This presents a serious difficulty, because at present there is no criterion for determining what is really a single disc. The discs range in thickness down almost to the limits of visibility and it is not known whether the thicker ones merely represent compounds of the thinnest ones, or whether some of them may themselves be really single. This feature will be illustrated in connection with some of the examples considered below.

Further difficulty is presented by the wide range of variation in the structures under consideration, not only from specimen to specimen, but sometimes even in the same gland (METZ 1937 and earlier papers). The variation appears to be due to differences in relative distribution or arrangement of the chromatic and achromatic materials, but its significance is not clear. One possibility is that a considerable amount of achromatic material is normally diffused through the chromatic material of the discs

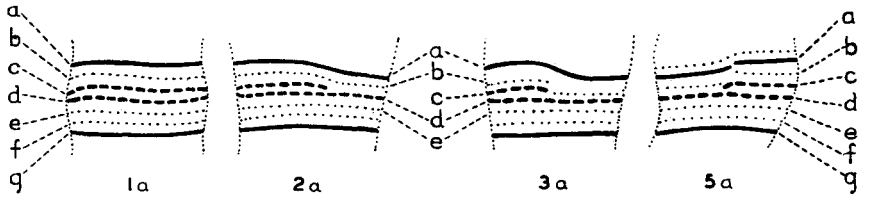
* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.




FIGURES 1–11.—Photographs of portions of giant salivary gland chromosomes in *Sciara ocellaris* Comst., illustrating various deficiencies. From aceto-carmine smear preparations. 1–5 and 9 from fresh preparations; the others from permanent mounts made by removing the covers in dioxan and mounting in damar. Magnifications approximately as follows: 1, 1690×; 2–8, 1920×; 9, 2800×; 10, 11, 1920×.

FIGURES 1–5, end 2 of chromosome A illustrating the first example described. 2 and 3 from one specimen; 4 and 5 from another. Compare with the corresponding diagrams in figures 1a, 2a, 3a, 5a.

FIGURES 6–8, end 1 of chromosome A illustrating the fifth and sixth examples described; all from one specimen; 8a and 8b from the same chromosome, at different focal levels. 8b is included




FIGURES 1a, 2a, 3a, and 5a.—Diagrams representing conditions in the corresponding photographs in figures 1, 2, 3 and 5. In 1a bands c and d are present in both homologues; in the others one of them, presumably c, is absent from one homologue. Note that the achromatic zones b–c and d–e on the non-deficient side are like b–d and d–e on the deficient side. In these diagrams bands b, e and f are too light. No significance should be attributed to the number of dots in any of these bands.

and that under different conditions, either in the living state or during fixation, the two separate out in different ways giving different details of pattern. This presumably does not affect the major pattern formed by the linear series of conspicuous discs, but it does complicate the problem of determining what represents a single disc.

The best approach to the present problem as a whole seems to be through comparative study of several small deficiencies, ranging down to the smallest. For this purpose Sciara is particularly favorable because here such deficiencies may readily be found in ordinary stocks without irradiation or other special treatment. Several examples are considered in the present account, which is based entirely on cytological evidence. None of the structures described has yet been identified with any particular genetic character. Some of the deficiencies appear to be common and widely distributed in nature, for they have been found in stocks secured from both North Carolina and Alabama, and at intervals of more than three years. We are indebted to Dr. J. PAUL REYNOLDS for these strains. All the material considered here is from *Sciara ocellaris* Comst.

As will appear below, the general trend of the evidence from these deficiencies seems to indicate that any disc, down at least to the small, thin ones, is divisible into two or more discs sufficiently independent so that one may be lost or acquired without the other. Where the ultimate limit is reached is not yet clear.

## FIRST EXAMPLE

The first deficiency to be described is particularly interesting because of the nature of the locus in question and of its immediate surroundings. The

---

to show the nature of the band at *a* in the non-deficient homologue. This band is partly out of focus in 8a.

FIGURE 9, for comparison with figures 17–20 (see text). Figure 10, portion of chromosome B showing the seventh example described. Figure 11, portion of chromosome B showing the eighth example. For details of all figures see text.

case will be considered in some detail and will be used as a basis of comparison in considering the others. The region is located near end 2 of chromosome A, and the deficiency appears to involve only a single band, as indicated by the photographs shown in figures 1–5. The non-deficient condition is shown in both homologues of the chromosome pair in figure 1, at the point indicated by the dotted line. In this condition the region in question usually exhibits a double "band," or a pair of apparently single bands, flanked on either side by a very thin or light band (at b and e in the accompanying diagrams). Such a grouping is especially favorable for present purposes, as will be indicated below. The double band or pair often appears as shown here, in the form of a wafer-like structure, or narrow "capsule," the two faces of which appear as single bands or rows of granules. In this case the wafer or capsule may enclose a single transverse row of achromatic droplets. In specimens showing the deficiency the non-deficient homologue exhibits the condition just described, while the deficient one lacks one element of the double band or pair, as shown in figures 2–5 and 2a–5a.

Three features of special interest are brought out in this case. (1) The deficiency apparently involves a single chromatic band. (2) The achromatic zones or bands at the left and right of the single chromatic band in the deficient chromosome (above and below in the illustrations) are apparently just like the corresponding ones at the left and right of the double band or pair in the other chromosome. (3) The achromatic material between the two bands of the pair in the latter chromosome appears to be entirely absent from the deficient chromosome. In a descriptive sense at least, the unit difference between the two chromosomes is one chromatic band and the achromatic material between the two bands. So far as visible evidence goes the loss (or acquisition) of a chromatic band here is accompanied by a modification of the achromatic constituent on one side but not on the other. This may be interpreted in two or more ways as noted previously (METZ 1937). It may be assumed that the chromatic disc and the achromatic material on one side of it form a unit. Or it may be assumed that a regulating factor operates to determine the amount of achromatic material between any two given discs. The converse could, of course, be postulated by assuming that the achromatic material determines the nature of the adjacent chromatic discs.

In earlier papers attention has been called to the fact that the achromatic material in these chromosomes is present in the form of droplets giving an alveolar or honeycomb type of organization, modified, of course, by the stratification due to the chromatic discs. Each two successive chromatic discs are separated by a row or transverse plate of achromatic droplets, which may be thick-walled or thin-walled according to the amount of

chromatic material surrounding them. Although not subscribing to this interpretation of chromosome structure, BAUER (1935 and 1936) has called attention to large heavy-walled droplets in Chironomus and evidently considers them as structural units (chromomeres). PAINTER and GRIFFEN (1936) have likewise emphasized such structures in Simulium. The latter authors apparently consider the chromosome as composed of numerous chromonemata each of which includes a series of such droplets ("chromomeres") aligned on a delicate thread. The question of the significance of the droplets and whether they represent parts of chromonemata has been discussed elsewhere (METZ 1937, METZ and LAWRENCE 1937, and earlier papers) and need not be reviewed here except as noted in the discussion.

For present purposes the achromatic material between two successive chromatic discs may be considered as a unit. The immediate problem is that of ascertaining the actual relationship between this material and the adjacent chromatic material. Several possible interpretations suggest themselves. These differ largely in the relative significance attributed to the chromatic and achromatic materials. If the chromatic discs represent the genic material and the achromatic interspaces are merely composed of non-genic connecting material, then attention may be confined mainly to the former, and the significant unit is the disc. If, however, the achromatic material is considered significant, the essential unit should be achromatic or should be represented by a combination of achromatic and chromatic materials. It is difficult to consider the unit as asymmetrical and composed of a chromatic disc combined with the achromatic material on one side of it. This would presumably require a regular orientation of all units, which is opposed by the evidence from inversions. An alternative interpretation would be that chromatic and achromatic materials are combined to form a unit, but that such a unit consists of three layers instead of two. It could consist of a chromatic disc lying between two layers of achromatic material, or of a layer of achromatic material lying between two layers of chromatic material. In either case the unit would be a wafer-like structure in which the two faces differed from the interior.

Several lines of evidence suggest that the giant chromosome might be made up of such wafer-like units in the manner indicated schematically in figure 12. We have attempted to apply hypotheses of this kind but have not yet found one which shows a satisfactory agreement with the evidence. Brief attention may be given, however, to one such hypothesis which appears to come closest to fulfilling the requirements at present. It is the last one mentioned above, illustrated in figure 12.

On this hypothesis the chromosome may be considered to be composed of a series of closely appressed wafer-like segments, each segment composed of a plate of achromatic material or droplets, the two faces of which

are covered by a layer of chromatin. On this view a chromatic disc would represent the interface between two closely appressed segments and be composed of the chromatin of these two appressed faces. On such an interpretation a single segment deficiency should not involve the entire loss of a single chromatic disc but rather the loss of an achromatic plate together with part of each of the two adjacent discs. A break in the chromosome, therefore, should occur within a chromatic disc rather than between discs. It would be reasonable to assume on this view that the two faces of the hypothetical segment should be alike. Two lines of evidence should be particularly important in testing this hypothesis. It would be expected, on the one hand, that heavy bands or discs would not appear

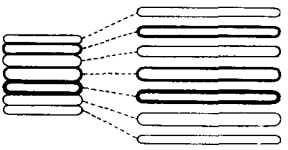


FIGURE 12.—Diagram illustrating one hypothesis of chromosome structure considered in the text This hypothesis is not supported by the evidence.

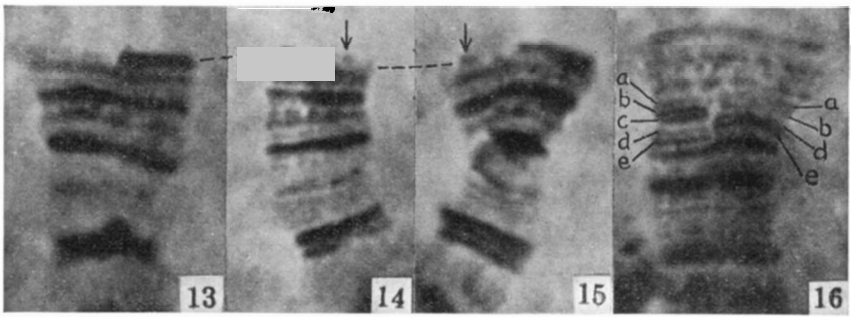
singly, because if one face of the segment is heavy the other one should likewise be heavy. Unfortunately, the evidence on this point is not conclusive because, although apparently single heavy bands may be observed, it is not yet possible to make certain that they are actually single.

The second line of evidence is that provided by cases such as the one described above. It involves a deficiency in a region having a moderately heavy double band or pair flanked on either side by very light bands. The two bands of the pair should represent the two faces of one wafer or segment. If this segment is lost, the two bands should disappear and leave one light band representing the faces of the two adjoining segments. Apparently, however, this does not occur, for the remaining band (d, in figures 2a, 3a, 5a and photographs) is heavier than would be expected on this basis, and appears to correspond to its apparent mate in the other chromosome. To explain the results on the hypothesis under consideration, therefore, would require the assumption that the two faces of the hypothetical segment are not alike, and there seems no legitimate ground for making this assumption at present. The evidence seems best interpreted on the assumption that the chromatic disc is lost or acquired as a unit, unless we assume that the two component discs under consideration are not single. If each of them is itself a pair, then of course the situation is entirely different; but, as will be shown below, deficiencies of this general type are found which involve the loss or acquisition of "single" delicate bands that are almost as small as the smallest which can be detected. The

evidence is strong that they are really single and require the interpretation just given.

## SECOND EXAMPLE

The second case is interesting in connection with that just considered because it apparently involves the same general type of original structure, but a different type of loss. It is a terminal deficiency, at end 2 of the same chromosome. In the non-deficient condition (figure 1) this chromosome terminates in a structure which may appear as a single heavy band, a double band, a pair of bands, or a small "capsule" inclosing a single transverse row of achromatic droplets. The deficiency in the present case, however, involves the loss of both members of the pair instead of only one, as indicated in figures 13–15. These figures are all from one specimen. The glands were fixed in formalin, followed by 45 percent acetic acid, and stained in crystal violet.




FIGURES 13–16.—Photographs illustrating the second and fourth deficiencies described in the text. 13–15 from a formalin-acetic-crystal violet preparation, 16 from the same preparation as figures 6–8. All approximately 2350×. 13–15, end 2 of chromosome A; 16, end 2 of the X chromosome. See text for details.

In the present case it is not clear as to just what is lost in addition to the pair of chromatic bands. At first sight it looks as if the adjacent achromatic material was not lost, and that the deficient homologue terminates in a zone of achromatic material. This is suggested especially by figure 13. It seems probable, however, that this is not the case, and that the terminus here is a very delicate chromatic band which is not visible in the non-deficient chromosome. Such a band is evident in this position in some preparations where no terminal deficiency is involved.

Certain characteristics of this delicate terminal band in the deficient homologue should be noted at this point because of their bearing on the question as to the significance of the chromatic "granules" in the giant chromosomes. In some cases, such as those shown in figures 14 and 15, most of the chromatin in this delicate band, or disc, has apparently ag-

gregated into one or two small lumps or "granules" (indicated by arrows). These show the same characteristics as the "granules" in other regions, yet it seems clear that they cannot represent individual genes in accordance with the interpretation advanced by some authors (for discussion see METZ 1937, METZ and LAWRENCE 1937).

### THIRD EXAMPLE

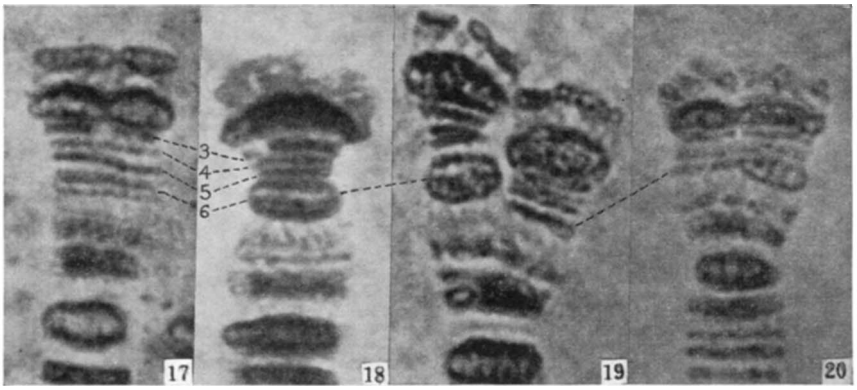
A third deficiency in this same chromosome appears to present a combination of the characteristics seen in the other two. In the non-deficient condition the structure under consideration apparently includes two similar double bands, which usually combine to form a large capsule; and the "loss" apparently involves an entire double element. The region is near end 1 of chromosome A, at the locus marked 6 in previous illustrations (METZ 1935a, figures 4 and 5). This region has been studied in hundreds of cells, including many examples of each of the three possible types, homozygous non-deficient, homozygous deficient and heterozygous. It is not known which condition is to be considered typical for the species. Many specimens exhibit at this locus what appears to be a single band or row of granules, which often forms a wafer-like structure or slender "capsule." In the latter case the single band or row of granules becomes transformed into a double band or two rows of granules, as indicated at 6 in figure 17 (also METZ 1935, figure 5, C). This condition has been discussed in another paper (METZ and LAWRENCE 1937). The adjacent band 5, which is typically a narrower band and often definitely a single row of granules (figures 17, 18, 20 and METZ 1935, figure 5, A, B) shows these same characteristics.

In these cases the differences in condition apparently reflect differences in the distribution of the achromatic in relation to the chromatic material. The "single" disc apparently becomes "double" by acquisition of achromatic material within it. In the resulting wafer-like structure each chromatic face looks like a disc. Both conditions may be found in one gland.

The structure just described, whether designated a single band, a double band or a pair of bands, evidently represents the homozygous deficient condition. In contrast, other specimens exhibit at locus 6 a much larger, heavy "capsule," as shown in figure 18, or two double bands each of which is as heavy as that just considered. This represents the homozygous non-deficient condition. Unfortunately, the strong tendency of this structure to form a capsule makes it very difficult to study the finer details of organization. It seems clear that the structure involves two relatively heavy double bands and intervening achromatic materials, as just indicated; but whether or not it also includes more than this is not certain. Apparently it does not.

The heterozygous condition is represented in figures 9, 19 and 20. As would be expected, the structures in the deficient and the non-deficient homologues correspond respectively to those just considered. The main characteristics are made clear by the fact that the structure on the deficient side may appear either as a single band, as in figure 20, or as two light bands or rows of granules, representing the two faces of the "wafer" or "capsule," as in figure 9. These two conditions may be found within the same gland.

It seems evident that the loss or acquisition here involves one double band or wafer, together with the achromatic material between the two.




FIGURES 17–20.—Photographs illustrating the third deficiency described. All represent end 1 of chromosome A; from fresh aceto-carmine preparations. 2500×. See also figure 9. 17 has been published previously (METZ 1935, Fig. 5 D). The numbering of the bands corresponds to that in figures 6–9 and in previous publications. In some cases, such as shown in figure 17, a narrow band, not numbered, is seen between 5 and 6, close to 6. For details see text.

As in the first example considered there is no apparent effect on the achromatic material to the left and right of the bands under consideration. The achromatic zones adjacent to the structure on the deficient side appear to be just like those adjacent to the larger, double structure on the non-deficient side.

In superficial aspects the present deficiency resembles the first example described, but actually the structures involved here all appear to be double as compared with those in the other case. In other words, the first example appears to represent the loss of one component of a double band, that is the loss of a single band; whereas in the present case it represents a loss of an entire double band.

### FOURTH EXAMPLE

This example involves a deficiency of the type seen in the first example, but the size of the structures is more like that of those in the second case

described. The region is located near end 2 of the X chromosome, as indicated in figure 16. On the non-deficient side there is a conspicuous double band, or pair (bc) while in the deficient chromosome, on the other side, there is a single band at the corresponding locus (b). In this particular nucleus, at this focal level, the two adjacent bands d and e are not continuous across the chromosome, and their appearance suggests the possibility of an inversion. This is not the case, however, as shown by other nuclei, where they are continuous.

Aside from the fact that this example exhibits characteristics in conformity with those already discussed, it is of interest because it may possibly involve loci responsible for sex determination. As indicated in earlier papers (METZ 1931, METZ and SCHMUCK 1931) there are two kinds of X chromosomes in *Sciara coprophila*, one designated X and the other X-prime (X′). Females homozygous for X are male-producers, while those carrying X′ are female-producers. Comparable conditions appear to exist in the present species, and the widespread occurrence of the chromosome condition described and figured here suggests that it may represent the X′X condition. This possibility is being investigated.

### FIFTH EXAMPLE

This example is essentially like the last and needs little description. It is shown in figures 6–8 at the locus marked *b*. The deficient homologue possesses a narrow band (fig. 6) or row of granules (figs. 7 and 8a), while the non-deficient one possesses a double band apparently composed of two of the single ones. This case is included because it accompanies a smaller deficiency, next to be described. The two are found near end 1 of chromosome A, the former at the point designated 13 and the latter between points 10 and 11 in previously published photographs (METZ 1935, figure 5).

### SIXTH EXAMPLE

This case possesses special interest because of the small size of the structures involved. They are only about half the size of the smallest ones considered above, as shown at a in figures 7 and 8. The band on the non-deficient side, shown at the left in the figures, usually appears single. But it is clearly double in one cell of the specimen from which these photographs were taken. It presumably represents two closely approximated, very narrow discs. On the opposite side only a single dotted line appears, which is often so faint as to be barely visible. Apparently it represents one of the two delicate discs going to make up the band in the other homologue, for it connects with the double structure and shows the ordinary indications of homology.

This deficiency involves a structure almost as small as any which can be examined satisfactorily, and it seems highly probable that it represents a "single" disc if this term has any significance in more than a descriptive sense.

SEVENTH EXAMPLE

Like the case just described, this one involves very small structures. It is found a short distance from end 1 of chromosome B, and is represented at a in the photograph shown in figure 10.

The deficient homologue, at the right, shows a delicate band or row of delicate granules, and the non-deficient member shows at the same locus a narrow band which apparently represents two delicate discs, one of which is presumably the counterpart of the single one on the other side. Here again the difference involves a disc about as small as any which could be used with confidence in such a study.

EIGHTH EXAMPLE

The present case is essentially like the two preceding ones, but the structures are somewhat larger, as shown in figure 11, at bc. The band at d is continuous across the chromosome in other cells, showing that the condition does not represent an inversion. This deficiency also is found near end 1 of chromosome B, but is closer to the end than is the preceding one.

*Discussion*

Small deficiencies visible in the salivary gland chromosomes have been described by several investigators, including MACKENSEN (1935), MULLER (1935), MULLER and PROKOFIEVA (1935), DEMEREC and HOOVER (1936) and EMMENS (1937), but so far as I am aware no detailed evidence has been presented bearing on the particular aspect under consideration in the present paper. Two of the three drawings shown by EMMENS to illustrate the deficiency known as "roughest$^2$" (his fig. 3) in *Drosophila melanogaster* represent a large achromatic zone in the deficient chromosome opposite the two prominent bands in its mate. Taken alone, these would suggest that in this case loss of chromatic bands was not accompanied by loss of achromatic material. But in the third drawing no achromatic zone is shown at the point of deficiency; the gap is completely closed. This fact, together with the author's statement that the chromosome is usually buckled at this point and that good figures are difficult to secure, makes it probable that the gap in the first two figures is due to stretching the deficient homologue and that the actual condition agrees in this respect with that described in the present paper.

The present account is mainly concerned with three questions: (1) What is the smallest unit which can be detected through study of small defi-

ciencies in the giant chromosomes? (2) What is the composition of this unit in terms of the visible chromatic discs and achromatic materials? (3) What light do the results of such a study throw on the nature and relationships of the chromatic discs and the achromatic materials?

Eight deficiencies have been described, ranging from cases involving two or more discs, down to others involving apparently single, very small discs. The latter appear to be almost as small as the smallest detectable discs. The study is not concerned with the genetic effects of any of the structures, but only with morphological aspects of organization.

Each of the "single band" deficiencies represents the loss or acquisition of one element of a double band or pair of bands. Whether or not the two are alike, or duplicates, in more than a morphological sense, is not known, but, as noted below, it seems probable that they are. The "unit" in each of these cases includes what appears to be a single disc, and also, in a descriptive sense at least, the achromatic material between the two similar discs. The loss or acquisition of a disc is apparently accompanied by loss or acquisition of achromatic material; but whether or not the two materials are actually combined in a definite manner to make a unit is not clear. This question is discussed in some detail in connection with the first example described.

The bearing of these findings on the interrelationships of chromatic and achromatic materials, and on the nature of the discs, is also discussed to some extent in the section just referred to. There are certain additional features, however, which may be noted in this connection. Attention has been called by several investigators, including BRIDGES (1935), BAUER (1936) and PAINTER and GRIFFEN (1936), to double, scalloped bands or to pairs of bands which may in some cases appear as single rows of conspicuous, heavy walled droplets or as wafer-like structures of the type described above. These droplets have been considered as discrete structural units, or chromomeres capable of reproduction, especially by BAUER and by PAINTER and GRIFFEN. On the view of PAINTER and GRIFFEN the pair of bands (discs) and intervening material represent a transverse plate of chromomeres and hence a unit locus. The structure should, therefore, behave as a unit; neither disc could be lost or acquired without the other. These authors apparently apply this conception to double bands in general, including the type considered in the present paper.

The cases described above involving a difference of one band of a pair provide strong indication that double bands or pairs do not represent units, and that consequently the single row of droplets, often visible in these cases, is not made up of unit chromomeres. This interpretation is given weight not only by conditions in the individual cases, but by the fact that they all agree, and that the range extends down to such small bands.

So far as the evidence goes it seems to indicate that each detectable disc is essentially independent, in the sense of being separable from its neighbors. If this is true it is difficult to attribute significance as genetic units to the achromatic droplets, either thick-walled or thin-walled. This agrees with the inferences drawn from other characteristics exhibited by these droplets, as indicated in earlier papers cited above.

The question might be raised as to whether or not, in the cases treated here, the single band in the deficient homologue is really homologous to one element of the double band in the other homologue. If it were not, the deficiency in each of these cases would be a deficiency of two discs instead of one, and the interpretation might be distinctly different. The evidence seems clear on this point, however, and seems to show that the homology exists, as described. This is indicated not only by the continuity of the structure across the double chromosome in many cases, but also by the similarity of conditions in the different cases.

The fact that in each case of a "single band" deficiency the single disc on the deficient side is matched by two similar discs on the non-deficient side suggests strongly that the cases involve duplication (acquisition) of a disc, rather than loss. This tends further to support the interpretation of homology just mentioned.

The evidence from these deficiencies agrees with that from most studies on the giant chromosomes in pointing toward the chromatic rather than the achromatic materials as the genetically important constituents. Both may exhibit considerable variation in appearance, but apparently the variation in the chromatic discs is largely due to variations in the amount and distribution of achromatic materials.

## SUMMARY

Eight small deficiencies are described (in Sciara) in which one homologue possesses structures not present at the corresponding locus in the other.

Two involve the loss or acquisition of a double chromatic disc or pair of discs; the others involve only one member of such a double structure. The smallest ones are so small as to make it highly probable that they represent really single discs.

In descriptive terms, the smallest unit loss or acquisition involves one chromatic disc and the layer of achromatic material on one side of it, but it seems improbable that the unit is really made up in this manner.

The bearing of the findings on some of the current hypotheses of chromosome organization is discussed, and it is concluded that the heavy walled droplets, described as representing unit genes or chromomeres by some authors, probably are not unit structures and do not represent unit loci.

## LITERATURE CITED

BAUER, H., 1935 Der Aufbau der Chromosomen aus den Speicheldrüsen von *Chironomus Thummi* Kiefer Z. Zellf. mik. Anat. **23**: 280–313.

1936 Beitrage zur vergleichenden Morphologie der Speicheldrüsenchromosomen. Zool. Jahrb. **56**: 239–276.

BRIDGES, C. B., 1935 The structure of salivary chromosomes and the relation of the banding to the genes. Amer. Nat. **69**: 59.

EMMENS, C. W., 1937 Salivary gland cytology of Roughest$^3$ inversion and reinversion, and roughest$^2$. J. Genet. **34**: 191–202.

METZ, C. W. 1931 Unisexual progenies and sex determination in Sciara. Quart. Rev. Biol. **6**: 306–312.

1935 Structure of the salivary gland chromosomes in Sciara. J. Hered. **26**: 177–188.

1937 Deficiencies and structural variations within the giant chromosomes in relation to the problem of gene structure. Proc. Nat. Acad. Sci. **23**: 137–142.

METZ, CHARLES W., and SCHMUCK, M. LOUISE, 1931 Studies on sex determination and the sex chromosome mechanism in Sciara. Genetics **16**: 225–253.

METZ, CHARLES W., and LAWRENCE, ELIZABETH GAY, 1937 Studies on the organization of the giant gland chromosomes of Diptera. Quart. Rev. Biol. **12**: 135–151.

MACKENSEN, OTTO, 1935 Locating genes on salivary chromosomes. J. Hered.—**26**: 163–174.

MULLER, H. J., 1935 A viable two-gene deficiency. J. Hered. **26**: 469–478.

MULLER, H. J., and PROKOFIEVA, A., 1935. The individual gene in relation to the chromomere and the chromosome. Proc. Nat. Acad. Sci. **21**: 16–26.

PAINTER, T. S., and GRIFFEN, ALLEN B., 1936. The origin and structure of the salivary gland chromosomes of *Simulium vergatum*. Records of the Genetics Society of America **5**: 202–203.