SALIVARY CHROMOSOME ANALYSIS OF THE WHITE-FACET REGION OF DROSOPHILA MELANOGASTER

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THE white-facet region of the X chromosome has been utilized extensively in both genetic and cytological studies. It is interesting genetically because of the unique series of alleles recognizable in the white locus and because of the series of alleles and the dominance of the deficiency for the facet locus. It is interesting cytologically also because of the grouping of heavy bands visible in salivary gland chromosomes. In this paper, the results of genetical and cytological studies of fourteen deficiencies in that region are reported.

Except for N-8 (MOHR 1923), 264-36, 264-37, 264-38, and 264-39, the material for all other deficiencies and the data for a number of genetic tests were obtained from M. DEMEREC. N-8 (MOHR) and 264-38 originated spontaneously while all other deficiencies were induced by X-ray treatment. Critical salivary chromosome figures were checked by C. B. BRIDGES, M. DEMEREC and B. P. KAUFMANN. Photographs and redrawing of figures were done by Miss R. W. PARKER.

METHODS

In order to obtain Notch deficiencies, normal males carrying yellow (y—0.0) as a marker were irradiated with approximately 2500 r-units, and then were mated with females homozygous for cherry (w^{ch}—1.5) and wavy (wy—40.7). From F_1 females the white or Notch flies were selected for this work.

H. BAUER'S (1936) method for preparing salivary glands was followed. Full grown larvae were dissected in aceto-carmine; needles and forceps of stainless steel were used to avoid contamination by iron. Slides were made permanent by the alcohol-euparal technique. Microscopical observations were made using a 2 mm. 1.4 N.A. apochromatic objective, an oil immersed achromatic 1.4 condenser, and 12.5× or 15× compensating oculars. A Bausch and Lomb research lamp equipped with a green Wratten filter No. 62 was used as a source of light. Drawings were made with the aid of a camera lucida.

Non-synapsed chromosomes were selected for study, because even good preparations of the region in question usually show such distortion that identification of bands is very difficult. In non-synapsed chromosomes the number of bands involved was determined and the length of the deleted

part was found by comparing the deficiency with a normal haploid strand. In many cases the bands of this region were identified easily by their characteristic size and shape, as well as by the intensity of their staining. To reduce any possibility of error, however, the limits of the deficiency were determined also in the synapsed condition of the chromosome. In that case, the exact position of some deficiencies was determined by combining them with the 258-18 translocation, in which the tip of X chromosome to 3C4 inclusive is transferred to the spindle fibre region of the fourth chromosome, exchanging places with the remainder of the fourth (DEMEREC and SLIZYNSKA 1937). This gave a clearer picture of synapsis in the one end of the deficiency and at the same time it usually prevented synapsis at the other end. But all these methods of examination sometimes failed, especially when the studied band was very faint and lay in a very narrow space between two heavy darkly stained bands or vesicles.

<div align="center">RESULTS</div>

The results of both breeding and cytological studies are summarized in figure 1. For a detailed description, BRIDGES' salivary gland chromosome map and his reference system (BRIDGES, 1938) were used. According to this system, every vesicle is counted and numbered as two separate bands.

258-11. This deficiency, as shown by breeding results, includes only one known locus, white, and extends in its cytological picture from 3A3 to 3C2.3 inclusive. In this case it is impossible to determine whether the 3C4 band is present or not, because this band is so faint that it is rarely visible even in the normal chromosomes. It was seen only twice during this work. 258-11, therefore, involves fifteen or sixteen bands.

258-14. Genetically identical to the former, this deficiency is a little shorter cytologically. The first band involved in the deficiency is 3A4, and the last band deficient without any doubt is 3C1. However, the possibility that 3C2 is also absent has not been excluded.

N-8 (Mohr). Cytological data in regard to this deficiency differ in some respects from those which are given by MACKENSEN (1935) and GOTTS-CHEWSKI (1937). According to MACKENSEN, this deficiency does not involve 3C1, but involves the double band 3E1 and 3E2. On the contrary, GOTTSCHEWSKI shifts the limits to the left and places the deficiency from 3C1 to 3D4 inclusive. According to the data presented here, the deficiency extends from 3C1·2 to 3D5·6 inclusive. This determination is based on studies of both synapsed and non-synapsed strands. In haploid chromosomes with N-8 deficiency, between section 3B and 3E, only one dark band is present which may be 3C1 as well as 3E1·2. Although the general features of the band in question seemed to indicate the second possibility, additional preparations were made with the translocation 258-

18, to determine accurately the homology of this band. As is evident from the drawing and from the photomicrograph in figure 2c and 2d, the band in question synapses with one lying in the part of another haploid strand not affected by the translocation, namely, with $3E1 \cdot 2$. This gives critical evidence for limiting the size of N-8 deficiency to the dimensions stated above. Therefore, the N-8 deficiency includes 18 bands.

FIGURE 1. Genetic and salivary chromosome maps of white-Notch region of the X chromosome and a diagram showing results of the study of fourteen deficiencies of that region. Black areas represent deficient segments and shaded areas indicate sections for which it is uncertain whether or not they are deficient.

264-38. This is the largest deficiency described in this paper; it involves 45 bands, from 2D4 to $3E1 \cdot 2$ inclusive, and according to breeding results involves the loci *pn, w, rst, fa,* and *dm*. The females heterozygous for this deficiency show greatly reduced viability as compared with all other Notch cases, and even in good culture conditions they are distinctly smaller than other females. The drawing and photomicrograph figures 2k and 2l show the 264-38 deficiency as it appears in diploid condition, with the small loop formed by the normal haploid.

264-36. This deficiency involves the loci *w*, *rst*, *fa*, and *dm*. Cytologically it covers the section from 3A4 to 3D2 inclusive, containing 25 bands. A drawing and a photograph of that deficiency in a heterozygous condition is shown in figures 2a and 2b.

264-30. The photomicrograph in figure 2j shows a haploid strand of this deficiency. As is indicated on the corresponding drawing, figure 2i immediately following the group of dark bands 3A1 to 3A4 is a much lighter dotted line 3C9·10, not clearly visible on the photograph. The band 3C8 is probably present but since it was seen only in oblique light, this determination was marked as questionable on the diagram in figure 1. The deficiency includes 17 or 18 bands.

264-31. This deficiency, genetically, removes the loci *w* to *dm* inclusive. Cytologically, fourteen bands from 3C1 to 3D2 inclusive are missing.

264-32. This deficiency involves only the *rst* and *fa* loci. The left end starts between 3C2·3 and 3C5·6. The limit in regard to 3C4 is not determined exactly for the same reason as in the case of 258-11. The last band included in this deficiency of 3 or 4 bands is 3C7.

264-37, -39, -33, -2, -19. These five deficiencies are identical with one another in every respect. All were tested with *fa*, *fa^n*, *spl*, and *Ax* and showed the deficiency for them but not for other adjacent loci. Cytological observation disclosed in all these cases a short deficiency for only one band, 3C7. On photomicrograph figure 2f and drawing figure 2e, 264-39 is represented. The bands 3C1 to 3C5·6 appear as three dark, wide bands. The next band, 3C7, present in the left haploid does not have a corresponding band on the right side. The band 3C8 not well seen on the photograph is again present in both strands and the next two bands (3C9.10), which are clearly visible, are the last synapsed bands on this figure.

264-8. In this case, although breeding tests indicate that *fa*, *fa^n*, *spl*, and *Ax* loci are deficient, the cytological analysis did not disclose any visible deficiency. In homozygous condition, it produces a lethal effect not only for the whole organism but, according to the data obtained by DEMEREC (1934), it shows a cell-lethal effect in the hypodermal cells of females. The females heterozygous for the 264-8 deficiency show the whole complex of changes characteristic for other Notches. Since the region studied in this case consists of very dark bands usually fused together, the examination of single haploids will show best whether all bands are present. In order to obtain a higher number of non-synapsed strands and to identify the deficient chromosome, N-females of the genetical constitution N/dl-49, *y Hw m² g⁴* were mated with males carrying the 258-18 translocation. Of three chromosomes involved in this cross, one contains the dl-49 inversion, another the translocation X-4 so that the identification of the third chromosome carrying the change in the N-region does not

present any difficulty. The photomicrograph figure 2h and drawing in figure 2g, made from the preparation of female larvae from the cross described above, represent the section of a haploid strand of the N-chromosome. It shows clearly that all bands known for this region are present. Moreover, the distance between the single bands and the general appearance of the bands as to size, width, and intensity of staining, do not differ from those which were observed for the normal X chromosome.

<div align="center">DISCUSSION</div>

The cytological and breeding results are summarized on the chart of figure 1. On this chart a close relationship between genetical and cytological pictures of the deficiencies studied is evident, indicating that the deficiencies which are similar in their genetical features always have at least one band in common. This serves as a means for determining the position of various loci in salivary gland chromosomes. In this study the problem is limited to the region of the X chromosome from *pn* to *ec*. This section of the chromosome has also been studied by MACKENSEN (1935), GRÜNEBERG (1937), EMMENS (1937), and GOTTSCHEWSKI (1937). A comparison of the cytological results of different authors, however, presents difficulties because different reference systems are used in different papers.

The limits of the section in which prune (*pn*) is located are given by the left end limits of the 264-38 and 258-11 deficiencies. According to that evidence, the gene is located somewhere between 2D3 and 3A3. MACKENSEN's determination is closer because he designates for this gene the region 2D6 to 2F2 inclusive which probably corresponds on the revised BRIDGES' map to 2D3 or 2D4 to 2F2.

For the location of white (*w*-1.5) evidence was obtained from 258-14, *N-8*, and 264-31 deficiencies. *N-8* and 264–31 place this locus to the right of the 3B4 band, because 3C1 is the first missing band in both these cases. The 258-14 deficiency, which also includes the *w* locus, shows that this gene must be located to the left of 3C3 since this band lies beyond the limits of the deficiency. A number of figures examined during these studies indicate that 258-14 is not deficient for the 3C2 band, and if this is true, the band 3C1 represents the white locus.

According to GRÜNEBERG (1937) roughest (*rst*) is located 0.2 to the right of *w*. The same author states that *rst³* is associated with a long inversion. EMMENS (1937) determined both limits of this inversion, the left one being between 3C2·3 (designated by him 3C2) and 3C5·6 (designated 3C3) and the right in the inert region to the right of *bb*. EMMENS also studied *rst²* and described it as a deficiency for bands 3C4 to 3C7 inclusive. The deficiencies described here throw some light on this question. The deficiency 258-11, which does not include *rst*, shows that *rst* should
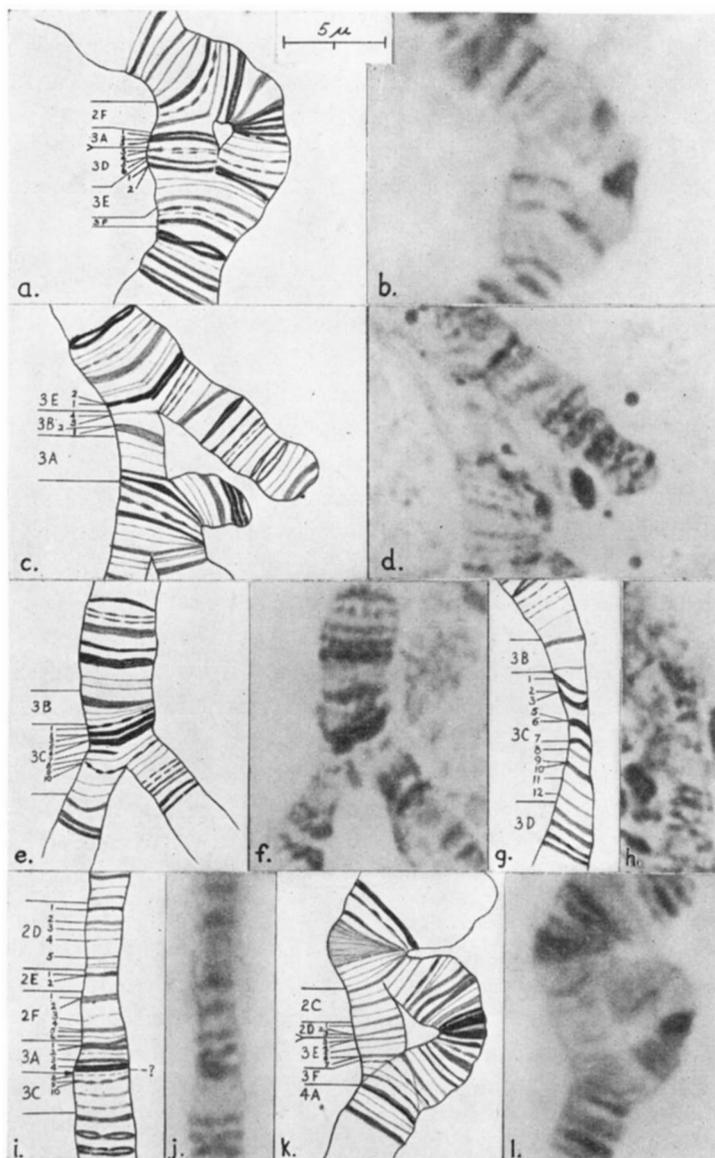
FIGURE 2. Drawings and photographs of various deficiencies. (a) and (b) 264–36 deficiency involving *w*, *rst*, *fa* and *dm* loci and cytologically a section of 25 bands, from 3D4 to 3D2 inclusive; (c) and (d) N-8 (Mohr) heterozygous with X-4 translocation 258-18 involving 18 bands, 3C1·2 to 3D5·6; (e) and (f) 264-38, one band deficiency; (g) and (h) haploid strand of 264-8 showing all bands; (i) and (j) 264-30 deficiency involving 17 or 18 bands, 3A5 to 3C7 or 3C8; (k) and (l) 264-38, the longest deficiency of this series involving 45 bands, 2D4 to 2E1·2.

be to the right of its right end, namely to the right of 3C2·3. The deficiency 264-32, which includes *rst* and *N* according to the genetical data, starts following 3C2·3 and includes 3C5·6 and 3C7, indicating that *rst* is located between 3C2·3 and 3C8. Deficiencies 264-33, -37, -39, -2, and -19 all of which include the 3C7 band but do not include *rst* show that *rst* is located to the left of 3C7, namely that it is represented either by band 3C4 or 3C5·6. Cytological analysis of translocation 258-18 (DEMEREC and SLIZYNSKA, 1937) shows that the break in the X chromosome occurred genetically after *rst* and cytologically between 3C2·3 and 3C5·6. Since 3C2·3 cannot represent *rst*, as shown by the deficiency 258-11, it seems probable that *rst* is represented by 3C4.

Facet, facet-notched, split, and Abruptex loci will be discussed together because their behavior in crosses with Notch is identical. It is known that notches are deficiencies for all these loci. The results of MACKENSEN place the locus for these genes at bands 3C4 to 3C7 or even probably 3C8. EMMENS locates it at 3C9·10, and GOTTSCHEWSKI put the genes *co*, *fa*, *spl*, and *Ax* in bands 3C5, 3C6, 3C7, and 3C8 respectively. The material used for our study includes five cases of notch deficiency, each including only the genes mentioned above. Cytologically they all show a deficiency for only one band, 3C7. If it is kept in mind that all cytologically known deficiencies occupying that band are, genetically, deficiencies for all these loci, it can be assumed that all these are represented by this one line. Therefore, there are two probabilities supported by cytological evidence: either these genes are allelic to each other, or one band covers several loci. Genetical data, however, are in favor of the first possibility.

The locus of diminutive (*dm*) should be placed to the left of the right end of 264-31 which includes it.

MACKENSEN (1935) has placed the position of the echinus (*ec*) locus in the region between 3E2 and 3E4. None of the deficiencies mentioned here includes *ec*, therefore, this locus must lie to the right of the right end of the longest of them, which is the 264-38; its righthand limit follows 3E2.

Notch 264-8 presents an interesting case of a genetical deficiency without any detectable change in the salivary gland chromosome. A similar Notch deficiency was first described by MACKENSEN (1935). To explain this phenomenon he assumed a dominant mutation with a Notch character. A second case studied in more detail was the Notch-G case described by GOTTSCHEWSKI (1937), who states that cytological examination showed a normal condition in the notch region. To explain the case, GOTTSCHEWSKI discusses three possibilities. First is the chain mutation hypothesis wherein gene changes have occurred in all the involved loci, producing allelomorphic forms, which, when crossed with respective recessive genes, give the illusion of pseudodominancy. Since this possibility requires several in-

volved assumptions he does not think it is probable. As a second possible explanation for removing the genes without the destruction of normal banding he suggests that some other agent might be responsible for the bands, which also seems improbable. He assumes, as the best explanation, the working hypothesis of inactivation, wherein the gene, although physiologically inactive, does not prohibit the formation of the band. For the present, no better explanation of the problem discussed here seems available.

Among the deficiencies studied, two are spontaneous and twelve induced. One of the spontaneous, 264-39, involves a single band; the other, N-8 Mohr, a block of 18 bands. Five induced deficiencies involve only one band while the remaining six cover larger sections. Although the number of cases studied is small, it appears that spontaneous deficiencies are similar to induced.

If the crossing over map for the region studied is compared with the approximate location of the genes in salivary chromosomes, an interesting fact may be observed. The distances between genes on the standard map do not correspond with those in salivary chromosomes. For example, the distance between y (0.0) and w (1.5) is the same on the crossing over map as that between w and fa (3.0). In the salivary gland, however, y is located at the tip of the X chromosome (M. DEMEREC and M. E. HOOVER, 1936) and w within the bands 3C1 or 3C2, so that the section limited by them is at least 12 times longer than the section between w and fa (3C7). It is difficult to determine what is responsible for this discrepancy. Between y and w there are about 75 bands, and between w and fa there are only about 5. The relation, therefore, is 15:1. The number of bands between y and pn is 57, if counted to the middle of the region ascribed for this locus, and the crossing over distance between y and pn is 0.8; between pn and w there are 18 bands and 0.7 crossing over units; between w and rst there are two bands and 0.2 crossing over units; between rst and fa there are two bands and 1.3 crossing over units. If we now calculate how many crossing over units correspond to one band in each segment separately we will obtain for y-pn, the value 0.014; for pn-w, 0.038; for w-rst, 0.1; and for rst-fa, 0.65. It is striking that the numbers are constantly increasing toward fa.

SUMMARY

Salivary chromosome studies of fourteen deficiencies affecting w and fa loci were made. The results are summarized in figure 1.

An approximate determination of the position on the salivary chromosome map for the loci involved was made. The locus pn (0.8) is placed between 3D4 and 3A2; w (1.5) is represented by 3C1 or 3C2; rst (1.7) by

3C4; *fa, spl,* and *Ax* (3.0) by 3C7; *dm* (4.5) is placed between 3C9 and 3D2 inclusive; and *ec* (5.5) to the right of 3E2.

One case of genetical deficiency without any visible effect in salivary glands chromosome structure is described.

The relations between the crossing over map and the salivary chromosome structure are discussed.

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