

# SALIVARY CHROMOSOME MAPS

With a Key to the Banding of the Chromosomes of *Drosophila Melanogaster*

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SINCE Heitz and Bauer have shown that the enormously enlarged chromosomes present in the nuclei of dipteran salivary gland cells are to be regarded as normal chromosomes rich in constant detail, and since Painter and his colleagues have demonstrated that the series of structures observable along the length of such chromosomes can be correlated to the series of genic loci on the linkage maps, it has become imperative for every *Drosophila* worker to make use of this new method of analysis. While the field of employment of salivary analysis is very wide, it is especially in those cases in which aberrations of the chromosomes may be involved, viz., deficiencies, duplications, translocations, inversions, etc., that the greatest saving in time and clarity of result may be expected. For such analysis two types of chromosome maps are prerequisites: first, linkage maps which give the sequence and locations of the genes for all mutant characters which may be involved, and, second, accurate detailed charts of all the normal salivary chromosomes against which to check the precise points of breakage or area of disturbance. A necessary adjunct to the detailed maps of the chromosome banding is an objective system of referring to a particular band or section of a chromosome. The general adoption of a system of "Salivary chromosome coordinates" by workers in this field is thus a matter of prime importance. The plan outlined below has proved in practice to have great advantages in being both accurate and elastic.

For many years I have maintained current linkage maps summarizing the genetic evidence on the locations of

genes. These have been published from time to time, and revised copies have also been sent to many individuals upon request. The latest revision will appear in a forthcoming number of this JOURNAL and will be distributed widely through the "Drosophila Information Service."

## The Wealth of Detail Observable in Salivary Chromosomes

Painter has just published in this JOURNAL (December, 1934, pp. 465-476) his survey of the normal salivary chromosomes, with maps which show the salient features. But, as he states, the amount of detail to be seen in the chromosomes is far in excess of that shown. During the past year I have attempted to make, for the structures of the normal salivary chromosomes, maps which would show this finer detail. Such detailed maps were found indispensable in precise study of chromosome aberration. They are herewith presented at a magnification sufficient to carry most of the detail, though to insure that the faintest lines observable be not lost entirely in reproduction, it has been necessary to sacrifice much of the range of intensities in the original drawings which were shown at the annual exhibit of the Carnegie Institution of Washington, December 14-17, 1934. Hence in using these maps it must be remembered that the fainter lines of the chromosomes and the fainter areas appear disproportionately too conspicuous and dark upon the maps reproduced herewith.

## Refinements Which Aid Observation

For observing the finest detail several refinements of technique are required.

One is relatively light transparent staining of the chromosomes, with avoidance of heavy "contrasty" staining, which may give the heavy lines very dark but the lighter lines not at all. Much iron and heating tend to spoil the finer details. The crispness of detail seen in larvae fully grown in pair cultures at a low temperature is lost in larvae from old cultures, from mass cultures and in larvae which have begun pupation. Especially favorable material has been attached-X (XXY) females of the race giant bobbed-11 picking giant larvae and examining the double-thickness chromosomes of certain cells found there.

Another requisite is selection of chromosomes or portions which are straight (i. e. not kinked or coiled) and are stretched somewhat. The lax chromosomes are from 70 to 110 times as long as normal gonial chromosomes, but the somewhat stretched chromosomes which are most favorable for observation are 150-160 times normal length. The maps presented herewith are drawn only from such partially stretched chromosomes, averaging 150 times normal. (For comparison a gonial group at the same magnification is included.) The gross structure of salivary chromosomes is somewhat like that of an accordian, and unless these chromosomes are stretched the doubleness of most bands is not visible and many fine or dotted lines are obscured by their appressed neighbors.

Too much attention cannot be paid to the illumination of the chromosomes. I use a 6-volt ribbon-filament lamp, and control intensity by a 175-ohm 2 amp. variable resistance in the 110-volt current to the transformer. The light from the ribbon is brought to a sharp focus in a small-area image about 30 cm from the filament and 25 cm from the mirror. This image is diaphragmed at that point to a circle the width of the ribbon image (3.5 mm). For turning the red color of the stain black, I set up Wratten filter 58A (deep yellowish green) behind one or more cobalt-blue glasses just before the mirror. A front-

silvered or aluminized mirror is preferable. An achromatic 1.3 or 1.4 condenser, oil-immersed, should be focused so that the edge of the filament image and/or the edge of the diaphragm are sharp in the field observed. The substage diaphragm should then be gradually closed down, with compensating adjustment of light intensity by the rheostat, until the area outside the image of the filament loses its light-haze and becomes dark, while the details of the chromosome banding increase in sharpness and contrast. Final checking should be made by oblique light (decentering the substage diaphragm) cast along the axis of the chromosome. I prefer 120 $\times$  apochromatic objective with 10 $\times$  compensating ocular for observations, but a 90 $\times$  with 10 $\times$  or 12.5 $\times$  eyepieces does nearly as well and does not require such critical attention to illumination, cover glass thickness and other details.

#### A System of Cataloguing Salivary Bands

The system of chromosome map nomenclature proposed in this paper divides the five main chromosome limbs (1=X, 2L, 2R, 3L and 3R) each into twenty sections, 100 in all. Sections are numbered 1 to 20 for X, 21 to 40 for 2L, 41 to 60 for 2R, 61 to 80 for 3L and 81 to 100 for 3R. Chromosome 4 has sections 101 and 102. Hence the number of a section is itself a key to the chromosome limb and to the relative position along that limb. Since sharpness and definiteness are essentials, each section begins with a conspicuous and easily recognized band. The division point is always made just to the left of the chosen main band, leaving all minor bands inside the division to the left of it. But since the 102 divisions average over 25 bands each, six subdivisions have been established for each division. Each subdivision also begins with a sharp band. They are designated by the capital letters A to F. A particular band would then be referred to as 17B3; a break would be referred to as just to the left

SCALE  $\leftarrow 5 \mu \rightarrow$ 

#### SALIVARY CHROMOSOMES AND GONIAL CHROMOSOMES COMPARED

Figure 4

Drawings of chromosome 4 of *D. melanogaster* and, on the same scale, of the entire group of gonial chromosomes at metaphase. In this gonial group the paired fourth chromosomes are represented by the small black dots, in which no structural details can be seen under the highest magnification, in striking contrast to the wealth of detail visible in the salivary chromosomes.

of 42C2 and a section as extending from 36A1 (included) to 38B1 (not included). The bands are not given definitive numbers on the maps because those numbers would change from year to year as our knowledge of the banding becomes more detailed and small bands are seen which were previously missed. No such change should be necessary for the divisions and subdivisions, since each begins with a sharp band already well established.

#### Landmarks of the Chromosomes

The "segmentations" observable in the chromosomes do not offer sharp enough boundaries for a serviceable reference system, though certain ones are very convenient landmarks for recognizing chromosomes in a tangle. Among the natural landmarks which should be learned first are the "puff" in 2B, the "four brothers" in 9A, the "weak spot"

in 11A, the two "chains" in 15, the "turnip" in 16 and the "offset" in 19E. The huge lightly staining nucleolus of the salivary gland cell nucleus is attached to the base of the X at the bands in 20C and D. Diagnostic of 2L are the "dog-collar" in 21CD, the "shoe-buckle" of 25A, the "shield" in 30A, the "goose-neck" in 31BF, the "spiral loop" of 32-35, a "turn-back" in 36, and the "basal loop" in 37-39. One recognizes 2R by its thick "onion" base and "huckleberry" tip. Three L has a "barrel" at 61CF, a "ballet skirt" in 68BC, "chinese lanterns" in 74-75 and "graded capsules" in 79CDE. Finally, 3R, the longest limb, has a large clear "cucumber" base 81-83D, a "duck's head" at 89E to 91A (frequently breaks at junction of 89D and E) and a "goblet" tip. Most of the apparent segmentation is inconstant and is due to the fact that the maternal and paternal part-

ners are sometimes seen side by side, as in section 25, and sometimes superimposed, as in section 24.

The free end of every chromosome limb presents a characteristically narrowed terminal region, seen especially clearly in the maps at 1A, 21A, 60F, but present also in part in 61A, 100F and 102F. It is suggested that this narrowed region represents a lag of one division in the gene-strings, somehow due to the terminal position occupied but not due to special properties of those particular genes.

### Size and Structure of the Chromosomes

The average lengths of the moderately stretched salivary chromosomes as drawn are: 1 = X = 220  $\mu$ ; 2 = 215 + 245 = 460  $\mu$ ; 3 = 210 + 275 = 485  $\mu$ ; 4 = 15  $\mu$ ; total = 1,180  $\mu$ . Thus the total length of the moderately stretched salivary chromosomes is approximately 150 times that of the total length of the gonial chromosomes, which is 7.5  $\mu$ . Individual salivary chromosomes with lengths exceeding 180 times normal have been measured. These excessively stretched chromosomes or portions show most of the stretching in the hyaline zones between the dark "bands." The heavier cross-bands or capsules retain their shape as broad firm discs while the material between may stretch into a narrow cord ten times its normal length but still showing its compound nature.

As deduced independently by Dr. Koltzoff (*Science*, Oct. 5, 1934) and myself (in press in U. S. S. R. since June, 1934) the large size of the salivary chromosomes is partly due to their being compound structures. Each of the fused maternal and paternal homologues consists of eight chromonemata or gene-strings derived from the corresponding chromosome of the gamete by successive divisions without complete separation of the division products. The cable of 8 + 8 strands shows its structure most clearly in the less heavy cross-bands in which 16 individual dots,

vesicles or small capsular units may be seen.

### The Relation of the Genes to the Bands

My inference as to the relation of the genes to the structures seen in the salivary chromosomes is that each of the faint cross-bands made up of 8 + 8 dots (see last line of 102D), dashes (middle line of 102E) or vesicles (first line of 102E) corresponds to one locus with 8 maternal and 8 paternal sister genes. I suppose that each of the sister genes of a locus is enclosed within one of the vesicular units, the gene itself being small and unstained but the walls of the enclosing capsular or clam-shell structure being visible from a deposit of chromatin. If the bipolar deposits of chromatin are somewhat heavier they run together at their edges to form two thin discs, one on each side of the plane of the genes. This hypothesis is in line with the observation of large numbers of thin bands (like those in 102A) which in edge view are wavy close doublets like the two halves of a split pea-pod. With still heavier deposits of chromatin, in amount characteristic for each locus, heavy obscurely double and somewhat nodulated bands result (like the first in 102B).

But besides these "thin-walled" structures there are "heavy-walled" structures (such as the first in 4F) which seem better interpreted as compound bands made up of two bands more or less united at their edges to give a "heavy-walled capsule." In very good preparations some of these heavy-walled capsules can be seen as two parallel bands not united at their edges. Certain of the heavy-walled capsules would seem to correspond to three loci, since they enclose between them a line of dots or dashes (4A1; 6A1; 7C1, etc.).

A count of the distinctly seen "lines" in the salivary chromosomes gave approximately 725 for X, 1,320 for 2, 1,450 for 3 and 45 for 4, totaling 3,540 lines. But a considerable proportion of the above lines seem incipiently double

or with an indistinct split. A count of "bands" or "loci," on the basis that each line of dots, dashes or vesicles and also each pair of closely approximated thin or moderately heavy lines represents one locus, while each heavy-walled capsule represents two (or three) loci, yielded: for X, 537; for 2, 1,032; for 3, 1,047, and for 4, 34 "bands." The total of 2,650 bands is in good agreement with calculations of 1,500 to 3,000 genes for the animal.

### Direct and Reversed Repetitions of Series of Bands

A structural feature of the highest theoretical importance is shown clearly in the "loops" and the "turn-back" in the basal half of 2L. Great difficulty was encountered at first in studying the base of 2L because of the troublesome kinks and coils. But in some cells the spiral coils were found stretched nearly straight and then it was observed that the edges of certain bands had been fused together and had been stretched out into connecting threads. The connected bands were found to match morphologically, 32F to 33C with 34F to 35C, in a whole series of repeated bands. The spiral loop was due to synapsis between homologous series of bands in two different positions in the same chromosome. Similarly, the basal loop in 37 and 38-39, when stretched out, revealed connecting strands linking (for example) the bands in 37EF and 38A with bands of identical morphology in 39CDE. Further study showed that all the bands in 37 were at least roughly matched in 38E to 39E (bracketed).

The "turn-back" in 36 with side by side fusion and the "shield" in 30A show reversed series forming sections symmetrical about their center points. It is significant that the bands in 30A show fusion along the surface of the chromosome to give a giant capsule.

This suggests that most of the larger capsules, such as the four-banded ones in 25A and in 56F, are symmetrical reversed repeats. The reversed symmetries around "weak spots" 3C and 11A may be further examples of duplications of sections of homologous bands.

How complicated a structure could be built by successive direct and reversed repeats is perhaps illustrated by the reversed symmetry with capsule tendency which centers around 33B and which is itself a part of the series repeated in direct sequence in 34-35.

The thick-walled capsules like that in 21E, some of which seem to enclose a line between them, as in 21D, may represent single-band or short repeats. There are also large numbers of pairs of lines of equal intensity, like that in 27E, the members of which are definitely separate. Perhaps some local process, such as unequal crossing-over, may have to be invoked to account for them.

### The Role of Duplications in the Evolution of Chromosomes and the Initiation of Species

For the long repeats, both direct and reversed, an origination as duplications through the process of translocation would seem an adequate explanation. In my first report on duplications at the 1918 meeting of the A. A. S., I emphasized the point that the main interest in duplications lay in their offering a method for evolutionary increase in lengths of chromosomes with identical genes which could subsequently mutate separately and diversify their effects. The present demonstration that certain sections of normal chromosomes have actually been built up in blocks through such "repeats" goes far toward explaining species initiation. For the duplication of sections of genes is known in *Drosophila* to cause many slight poorly-defined differences in all parts of the duplicant type.