PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 23 August 15, 1937 Number 8

GROWTH AND DEVELOPMENT OF THE SALIVARY GLAND CHROMOSOMES IN SCIARA

By John Bonner Buck*

William G. Kerckhoff Laboratories, California Institute of Technology

Communicated July 6, 1937

The chromosomes in the larval salivary glands of Diptera are unique in their enormous size, in the remarkable regularity and complexity of their visible detail and in the intimate fusion of homologs. The present investigation of *Sciara* is an attempt to follow these characteristics during the transformation of the ordinary small somatic chromosomes into the giant banded salivary chromosomes. Two methods were used. In the first, the growth of chromosomes, nucleus, cell, salivary gland and larva was measured *in vivo* throughout development. In the second, a careful study was made of the morphology of chromosomes fixed at each stage of development, in an attempt to correlate growth with morphological change. A detailed account of the investigation will be published elsewhere.

At room temperatures *Sciara* requires about a month for development from egg-laying to the emergence of the imago. Its four instars and pupation begin, respectively, at about 6.5, 10, 12.5, 15 and 24 days.

The salivary gland attains its definitive number of cells soon after it arises in the embryo within the egg, and its subsequent growth is due entirely to increase in cell size. In nucleus, cell, gland and larva, growth proceeds at a moderate rate up to the beginning of the second instar, then more rapidly until about the middle of the fourth instar, then more slowly until pupation, at which point visible degeneration begins and the size of each decreases.

In the early embryonic gland homologous chromosomes are somatically paired, but not fused in synapsis.[1] Each homolog is present as a relatively short and very slender thread lying parallel to its mate and about 0.4 μ distant, measured axis to axis. Each thread bears a small number of chromomeric enlargments corresponding to those on its homolog. Somewhat later each homolog splits into two threads and these sister strands are joined to each other by delicate cross-connections between some of the homologous chromomeres.

Shortly before the larva hatches from the egg the doubled homologs become more closely associated and begin to twist or twine about each other, caduceus fashion. At the same time the cross-connections between sister strands become heavier, obscuring the original chromomeric structure, except in stretched regions, and foreshadowing the "banding" of the typical salivary chromosome. The bipartite homologs continue to draw closer to one another, and the twisting, as it grows tighter, forms a helical coil which is composed of the two homologs (each double) lying parallel to each other and apparently in contact. In many nuclei of this age each coiled pair of homologs is distinctly separate from the others and all are oriented (polarized) toward the same region of the nucleus, so that the gross orientation resembles that in late telophase. As soon as the homologs come in contact a fusion between homologous regions begins, in the sense that the median line dividing them gradually disappears. This fusion may properly be considered the beginning of the synapsis of salivary mates. The earliest synapsed salivary chromosome thus is flattened, four-stranded and helically coiled. Synapsis is substantially completed during the first instar, which is very much earlier than the stage at which, according to Painter,[2] it occurs in *Drosophila*. By the end of the first instar each synapsed pair of homologs appears as a slender cylindrical, much elongated strand, considerably convoluted but only irregularly coiled. They stain lightly, show diffuse cross-bands at intervals and are considerably longer in proportion to their diameters than they are in old larvae.

Early in the second instar the chromosomes begin to increase in diameter and the banding becomes more pronounced. By the time the late second instar has been reached their structure, except for smaller size and fewer bands, is essentially that seen in old larvae. In this stage also, a regular coiling of each synapsed chromosome pair as a unit again begins to become prominent.

During the third instar the chromosomes increase in diameter at a constant rate. Midway through this instar the coiling reaches its maximum, and in favorably smeared preparations or in Feulgen mounts, each chromosome is seen to be coiled into a fairly tight uniform helix which superficially resembles the metaphase spirals seen in liliaceous chromosomes. In the late third instar the coiling begins to relax, and the chromosomes become progressively straighter throughout the fourth instar.[3]

The fourth instar is the period of greatest growth of the chromosomes. In reference to the development of the banding, the evidence indicates that the first bands which appear represent the heaviest bands of the definitive chromosome and remain relatively unaltered during development except to darken or to separate into doublets. The new, light bands, which become visible as development proceeds, appear in the lengthening spaces between the heavier bands, rather than split off from them. In the late fourth

instar there are usually visible certain small regions where the homologs are partially or completely non-synapsed, but no separation of the synapsed chromosome into more than two units has been observed. There seems to be in *Sciara* no visible differentiation of the definitive chromosome into four "bundles of chromonemata" of the sort reported in *Drosophila* and *Chironomus* by Tiniakov,[4] and in *Simulium* by Painter and Griffin.[5]

During the pupal stage the larval salivary gland undergoes histolysis. The main outlines of the degeneration of the chromosomes seem to be as follows: decrease in volume due to loss of the achromatic ("matrix") material; the collapse of enlarged regions to form heavy-walled vesicles; the persistence of very heavy bands; and the disappearance of delicate bands and fine detail.

If the average nuclear volume in the early salivary gland of the embryo is expressed as unity, calculation shows that the salivary gland nuclei in larvae at the time of pupation are about 1750 times as large. The curve of increase in nuclear volume during development is approximately exponential from about 4.5 days to 17 days (i.e., log nuclear volume plotted against time is a straight line) and can be superimposed rather closely upon a curve of the general form $y = 2^n$ up to the value $n = 10$. This indicates that nuclear volume doubles every 1.25 days during the period from 4.5 to 17 days. The rate of increase after the 17th day is progressively less until pupation. Doyle and Metz[6] have shown that in the living condition the full-grown salivary gland chromosomes of *Sciara* occupy at least 90 per cent of the volume of the nucleus. It is therefore not unreasonable to assume that the chromosomes increase in volume at approximately the same rate as the nucleus. This does not necessarily mean that there is a regular doubling of some internal structure of the chromosomes every 1.25 days, as their growth (increase in volume) could conceivably be due, partly or entirely, to some other cause, such as intake of fluid.

Chromosome volume cannot be computed directly because it is impossible to measure accurately the lengths of the chromosomes during the early stages. The growth in volume per unit length, however, is given at once by the cross-sectional area at different stages, and this, when calculated from the measurements of chromosome diameter, proves to increase about 75-fold between synapsis and pupation. During the same period nuclear volume increases 300-fold. Assuming that the chromosomes occupy the same percentage of nuclear volume throughout larval development, the above results mean that chromosome length increases about 4-fold $\left(\frac{300}{75}\right)$ during this period. The total length of the mature relaxed salivary chromosome group is around 800 μ, and that of the metaphase group around 10 μ. The 80-fold difference between these lengths is thus made

up of the 4-fold increase subsequent to synapsis and a 20-fold increase previous to synapsis.

The measurements of Belling[7] on *Lilium* show that the "fully-extended" leptotene chromonema is 10 times the length of the metaphase group. Measurements made by the writer from the drawings of Kaufmann[8] indicate that the difference is of the same order of magnitude in *Drosophila*. Kaufmann[9] and others have suggested that the heavily staining chromomeric granules seen in the spireme stages of some ordinary chromosomes represent regions where the connecting thread (chromonema) remains tightly coiled in a tiny spiral (not to be confused with the coiling of the chromosome as a whole, described above). Genetic evidence makes it probable that the definitive salivary chromosome is comparable to a chromonema entirely uncoiled and extended at full length. It is questionable whether mere "uncoiling" of chromonemata can account for the enormous (80 to 100-fold) difference in length between the metaphase and definitive salivary chromosomes, as assumed by Muller.[10] Rather, it seems more reasonable to assume that the great increase in length is due partly to uncoiling and partly to actual growth in length of the chromonemata, provided, of course, that the chromosomes are indeed made up of chromonemata. It is difficult to ascertain what proportion of the increase is due to either of the above factors, but it is interesting to note that previous to and during synapsis (first instar) the chromosomes increase greatly in length, but little in diameter. This period might, therefore, be one in which the chromonemata lose their hypothetical coiling and become completely extended. In the latter part of the fourth instar, when specific regions of the chromosomes can be recognized, it is reasonably clear that increase in length is due to actual growth of the chromosomes, elongation of the interband portions accounting for most of the increase. The fact that the chromosomes elongate appears to vitiate the calculations of Hertwig[11] concerning the number of chromonemata present in the salivary chromosomes, since in his work only the nuclei were measured, and no allowance was made for increase in nuclear volume by chromosome elongation.

Koltzoff[12] and Bridges[13] have advanced the hypothesis that the definitive salivary chromosomes are probably bundles of gene-strings (chromonemata) derived by repeated longitudinal division of the chromonemata present in ordinary somatic chromosomes. These investigators, in addition, have reported that in *Drosophila* the number of granules visible in a band frequently appears to be a multiple of 2, most often 8, 16 or 32. On this view the number of chromonemata present would be equal to or greater than the number of granules separately visible on any band. Metz and his coworkers,[14] while not disputing the compound nature of the salivary chromosomes, have raised serious objections to the view that there is any identity

of the "striations" which connect granules along the lengths of the chromosomes, with the individual genonemes.

In order to ascertain the relation between the number of chromonemata theoretically to be expected on the basis of the above measurements, and the number of granules and striations visible in the salivary chromosomes, let us make the following assumptions: (1) The synapsed salivary chromosomes are solid cylinders of circular cross-section, having the same type of structure throughout a given transverse section.[15] (2) The chromosomes increase in cross-sectional area by longitudinal multiplication of chromonemata. (3) All chromonemata formed during development are the same size and distance apart as the original threads at synapsis.[16] If we accept these assumptions tentatively, the data on cross-sectional area of the chromosomes show that since each chromosome (pair of homologs) contains 4 visible threads at synapsis (area = 1), one would expect that there would be approximately 300 chromonemata visible in each full-grown chromosome (area = 75). Observation, however, does not support this expectation. The maximum number of granules clearly visible in any band was around 20 (Metz reports counting about 30). Similarly, at every stage of postsynaptic development the observed number of granules per band is very much less than the number of chromonemata to be expected if the above assumptions are correct, although many more granules than are seen should be easily visible if present. The observations, however, do not preclude the possibility that the visible striations represent compound bundles of incompletely separated chromonemata.

In conclusion, the discrepancy between observation and calculation means either that one or more of the above assumptions is wrong or that the finest visible striations and granules in the mature salivary chromosomes do not correspond to single chromonemata of the sort clearly seen in some ordinary chromosomes.

The author gratefully acknowledges his indebtedness to Doctor Calvin B. Bridges, who offered much helpful advice during the course of the investigation and carefully confirmed the main observations, and to Professor C. W. Metz, who suggested the problem and furnished the material.

[1] Painter (*Jour. Hered.*, **25**, 465, 1934) and Dobzhansky and Tan (*Zeits. f. ind. Abst.*, **72**, 88, 1936) have drawn similar distinctions between pairing and synapsis.

[2] Painter, T. S., *Science*, **78**, 585 (1933).

[3] The progress of coiling in *Sciara* does not appear to agree with that reported for *Drosophila*, in which Koller (*Proc. Roy. Soc.* (London), Ser. B, **118**, 371, 1935) maintains that the chromosomes are progressively *un*-coiling from the earliest stages. Koller, however, studied mainly "developing" cells in glands from *old* larvae.

[4] Tiniakov, G. G., *Biol. Zhurnal*, **5**, 753 (1935).

[5] Painter, T. S., and Allen B. Griffin, *Genetics*, **22**, 202 (1937).

[6] Doyle, W. L., and C. W. Metz, *Proc. Nat. Acad. Sci.*, **21**, 75 (1935); *Biol. Bull.*, **69**, 126 (1935).

[7] Belling, John, *Univ. Calif. Publ. Botany*, **14**, 335 (1928).

[8] Kaufmann, Berwind P., *J. Morph.*, **56**, 125 (1934).

[9] Kaufmann, Berwind P., *Am. Nat.*, **65**, 280 (1931).

[10] Muller, H. J., *Am. Nat.*, **69**, 405 (1935).

[11] Hertwig, Gunther, *Zeits. f. ind. Abst.*, **70**, 496 (1935).

[12] Koltzoff, Nic., *Science*, **80**, 312 (1934).

[13] Bridges, C. B., *Am. Nat.*, **69**, 59 (1935).

[14] Metz, C. W., *Jour. Hered.*, **26**, 177 (1935); *Ibid.*, **26**, 491 (1935); *Biol. Bull.*, **71**, 238 (1936); *Proc. Nat. Acad. Sci.*, **23**, 137 (1937).

Metz, C. W., and E. H. Gay, *Proc. Nat. Acad. Sci.*, **20**, 617 (1934); *Science*, **80**, 595 (1934).

Metz, Charles W., and Elizabeth Gay Lawrence, *Quart. Rev. Biol.*, **12**, 135 (1937).

[15] This has been observed by Bauer (*Zeits. f. Zellf.*, **23**, 280, 1935), in *Chironomus* and in *Drosophila* (*Proc. Nat. Acad. Sci.*, **22**, 216, 1936) and it appears also to be true in *Sciara*, but Yasui (*Cytologia*, **6**, 330, 1935) and Tiniakov[4] maintain that the salivary chromosomes in *Drosophila* are flat tapes, and Koltzoff,[12] Heitz (*Biol. Zbl.*, **54**, 588, 1934) and Muller[10] that they are hollow cylinders.

[16] In the definitive salivary chromosomes of *Sciara* the visible striations and granules in some regions do appear to be approximately the same size and distance apart as the original chromomeric threads, but in many other regions they are either more or less numerous.

* NATIONAL RESEARCH FELLOW IN BIOLOGY.

DEVELOPMENT OF EYE COLORS IN DROSOPHILA: RELATION BETWEEN PIGMENTATION AND RELEASE OF THE DIFFUSIBLE SUBSTANCES

BY BORIS EPHRUSSI[1] AND SIMON CHEVAIS

INSTITUT DE BIOLOGIE PHYSICO-CHIMIQUE, PARIS

Communicated July 10, 1937

1. *Introduction.*—Eye buds of the mutants vermilion (*v*) or cinnabar (*cn*) of *Drosophila melanogaster*, implanted into larvae of the mutant white (*w*) develop into eyes which are wild type in color. It has been previously shown that a similar modification of *v* and *cn* eyes implanted into wild type is due to the presence of two diffusible substances (the v^+ and cn^+ substances) in the lymph of the wild type hosts.[2] It must be concluded then that the same substances are present in the *w* mutant and that the partial or total absence of pigment in the mutants of the *w* series is due to the disturbance of a mechanism other than that which leads to the formation of diffusible substances required in the development of the wild type eye color.

It has also been shown[2,3] that eye implants of various mutants can produce and sometimes release the diffusible substances; this can be shown by