[3] For the development of the feather germ the reader is referred to Lillie, F. R., and Juhn, Mary, *Physiol. Zoöl.*, **5**, 124–184 (1932).

[4] After removing the skin ectoderm, the donor embryo is allowed to develop until the tenth day or later when its sex is ascertained.

[5] Warren, D. C., and Gordon, C. D., *Jour. Agri. Res.*, **51**, 459–470 (1935).

[6] For a similar effect produced by neural crest, see Dorris, Frances, *Anat. Rec.*, **70**, Sup. 3, 91 (1938).

[7] DuShane, G. P., *Jour. Exptl. Zoöl.*, **72**, 1–31 (1935).

CONCERNING THE ORIGIN OF THE POLYTENE CHROMOSOMES OF DIPTERA

BY KENNETH W. COOPER

DEPARTMENTS OF ZOÖLOGY, UNIVERSITY OF ROCHESTER AND COLUMBIA UNIVERSITY

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In view of the unprecedented rapidity with which new findings on polytene[1] chromosomes are being published, it may not be amiss to draw attention to certain aspects which are not very often considered. Polytene chromosomes are known to occur in many larval tissues of Diptera other than those of the salivary gland. They have been recorded in the nuclei of the fat bodies, hypodermis, intestine, absorbing cells of the midgut, muscles, malpighian tubules, tracheal cells and sporadic cells in the brain (Balbiani 1881, Carnoy 1884, Dawydoff 1930, Heitz and Bauer 1933, Geitler 1933a, etc.).[2]

The writer has found that *Drosophila* larvae, fixed in alcohol, sectioned and stained with Heidenhain's haematoxylin, not only show unmistakable polytene chromosomes in the nuclei of the cells of the salivary glands and malpighian tubules, but frequently carry them also in the fat bodies, gut, hypodermis, some muscle fibers and oenocytes. Indeed, it appears that the large nuclei of those cells fated to histolyze during pupational reorganization all contain polytene chromosomes.[3] Dawydoff (1930) has indicated that the occurrence of such polytene chromosomes is hardly to be accounted for by physiologic specialization associated with glandular activity, as Darlington (1937) and others have supposed.

Origin of Polytene Chromosomes.—In general, all insect larval tissues destined to undergo histolysis during metamorphosis appear to grow by an increase in the size of their cells rather than by cell division (Trager). In the Culicidae (Bogojawlensky, Trager, Berger), Muscidae (Pérez), Drosophilidae (Frolowa 1937, Poulson) and probably all other flies, virtually all of larval growth is effected by an increase in the size of the cells concerned rather than by an increase in their number.[4] Buck (1937), Geitler (1934a,

1937a, 1938) and Berger[13] have cytologically examined nuclei of cells which undergo such growth. They demonstrated that in these cases (Diptera and Heteroptera) polyploid nuclei[5] are resultant, and the important point for the present purposes lies in the observed increase of chromosome materials during nuclear growth.

It is of considerable interest, therefore, to note that Buck (1937) has found that polytene chromosomes of the salivary gland of *Sciara* undergo a regular increase in dimensions correlated with growth of the nuclei. Furthermore, the largest somatic nuclei in the present writer's preparations have the largest polytene chromosomes, and the size of the contained chromosomes is proportional to the volume of the nucleus. The smallest tissue nuclei appear to be "resting" or *energic* [15] nuclei. That the polytene chromosomes are of different sizes among the nuclei of the tissues and that the largest polytene chromosomes occur in the largest nuclei was indeed noted by Balbiani (1881). Clearly, then, the polytene chromosome grows with the growth of its nucleus.

Koltzoff (1934), Bridges (1935), Bauer (1935) and many others have suggested that the polytene chromosomes of Diptera arise by repeated doubling of chromonemata, forming "multivalent chromosomes." Marshak[18] has experimentally demonstrated such growth of the chromosomes by means of x-ray-produced deletions in the chromonemata bundle. Recent estimates of the number of strands composing the polytene chromosome of the salivary gland of late larval life all agree that the number is a large one (*scil.*—*Sciara*, about 300, Buck 1937; *Simulium*, 64–128, Painter and Griffen 1937; *Chironomus*, 350–400, Bauer 1936; *Drosophila*, 256–512, Hertwig 1935), whereas Nebel and Ruttle (1937) have presented reasons for believing that the mitotic telophase chromosomes of animals, as well as of plants, contain but four chromonemata. As no more than four chromonemata need be supposed to exist in the telophase mitotic chromosomes of Diptera, during larval growth there must be an enormous increase in the number of chromonemata in the chromosomes of polytene nuclei—an increase in chromosome materials.

As these polytene chromosomes occur in the greatly enlarged nuclei of the most varied tissues, similar conditions probably obtain throughout much of the growing larva. It seems likely that, considering the pertinent data, both the growth of the cells and the chromonemata increases are in some manner related. Consequently it is suggested that the nuclei of the growing larva repeatedly prepare for unrealized prophasic condensation. These preparations involve chromonematal duplications whose number is not diminished by subsequent mitosis. Whatever the impediment to mitosis may be, this block at a particular nuclear stage apparently is the cause of both larval growth by increase of cell size rather than cell number, and, in part, the formation of the polytene chromosome.

Following the last mitotic division of organ differentiation in the fly larva, a chromosome may be expected to exist in the nucleus in a relic coil (Darlington 1935a) of a fairly large number of gyres (viz., 12–16 for the *X* of *Drosophila melanogaster*). The chromosome probably would possess, as the work of Nebel and Ruttle (1937) suggests, but four visible chromonemata. However, during growth to the third instar there is an increase of possibly more than 300 chromonemata. This increase in the number of chromonemata or chromosome materials would have a marked effect upon the helix or relic coil of the chromosome.[6]

As the thread number of the chromosome is repeatedly increased by duplications of the component chromonemata, and as these chromonemata remain tightly bound together, the girth of the chromosome must increase. But the chromosome is of helical (not spiral) structure. As the girth of the line of a tight helix of given height is increased, so the number of gyres must be reduced. Briefly, the increase in the number, or size, or both, of closely bound and precisely juxtaposed chromonemata of the chromosome results in a force which will tend to uncoil the chromosome.

To this uncoiling force must be added still another, probably of chief importance. That a chromosome undergoing transformation to the polytene condition must grow in length has been pointed out by Heitz (1935), Metz and Lawrence[21] and Bridges.[16] Buck (1937), Painter and Griffen (1937) and Frolowa (1937)[17] have, in fact, shown that such growth occurs in *Sciara*, *Simulium* and *Drosophila*, respectively. This is obvious, for the length of the lax polytene X-chromosome of *Drosophila melanogaster* is from 140–200 micra (Bridges, 1935), whereas the mitotic metaphase *X*-chromosome is little more than 2 micra in length. The latter length must be the height of the helix into which the chromonemata are coiled at mitotic metaphase. Thus it may be calculated that if the polytene chromosome length were equal to that of the uncoiled chromosome, the mitotic metaphase chromosome would possess either (*a*) an enormous number of coils (as Muller, 1935, conceived) far exceeding (over 200) the conditions in any known organism, or (*b*) the chromonemata must undergo multiple or double coiling (also, Muller, 1935) for which there exists no evidence for animal mitotic chromosomes.[23] Therefore it is suggested that the uncoiling or opening of the helix of the developing polytene chromosome is produced by both increase in length and number of its component chromonemata.

Buck (1937) and Bridges[16] maintain that simple uncoiling of the "normal" chromosome will account for only about one-eighth to one-tenth of the length of the lax polytene chromosome of the salivary gland. The growth in length appears to be accounted for chiefly by expansion of intergenic regions low in nucleic acid content. The heterochromatic regions of the normal chromosomes, on the other hand, apparently undergo little

if any longitudinal growth in the formation of the polytene chromosome. Whether this is due to the dense accumulation of nucleic acids in the heterochromatic regions is problematic.

Pairing Forces of the Polytene Chromosomes.—The fact that polytene homologs are generally synapsed at the completion of their growth stages is possibly and even probably the result of at least two distinct phenomena, namely, the uncoiling of the chromosomes and the forces of somatic pairing.[7] Somatic pairing is held to be due to the mutual attractions of homologous loci,[8] and appears to be expressed to an extraordinary degree in the Diptera. Now chromosomes may be expected to synapse to the degree that homologous loci may be approximated to each other. In the closely coiled mitotic metaphase chromosome the number of approximating loci would be at a minimum, and would little exceed the number of gyres of the metaphase helix. However, it may be expected that more and more homologous loci of a chromosome pair may be brought together by their mutual attractions as uncoiling proceeds. Thus, with uncoiling as complete as can occur within the confines of a nucleus, somatic pairing may reach its zenith and result in actual synapsis of the homologous chromosomes serially along their length[9] (compare Beasely[12]). If this is true, the supposed relational coiling observed by Koller (1935) in the polytene chromosomes of the salivary gland is little more than the perpetuation of the residual twists in the uncoiling chromosomes at the time of their pairing.

It has been observed that homologous polytene chromosomes do not always synapse (Bauer, 1936b), or fail to synapse regionally (Geitler, 1934a). Darlington (1937), probably correctly, points out that failure of synapsis in some cases may be due to a delay in the approximation of homologs. In addition, Painter and Griffen (1937) have shown regional peculiarities in the polytene chromosomes of *Simulium* to be associated also with incomplete synapsis.

Darlington's Hypothesis of Pairing.—Darlington's precocity hypothesis was brought forth in support of his belief that chromosome pairing and synapsis at meiosis is, in part, dependent upon the univalency of the synapsing chromosomes. However, Nebel and Ruttle (1937), and others have advanced reasons for the belief that leptotene chromosomes are split at the time of synapsis. Furthermore, the intimate synapsis of the polytene chromosomes—multivalent in strand number—further stresses the fact that Darlington's view is by no means an established one (also, Beasely[12]). It has been shown by Geitler (1934a), Buck (1937) and Frolowa (1937)[17] that each incipient polytene chromosome is composed of more than one strand at the onset of synapsis with its homologue, and Painter and Griffen (1937) lean to the same view. There seems no evidence for Darlington's contention that the chromonemata are associated closely in pairs in the polytene bundle (Painter and Griffen, 1937). Fur-

thermore, Painter (1934b) pointed out that, in the case of triploid *Drosophila*, three polytene chromosomes come together and synapse along their length in the same intimate fashion as do the polytene homologs of diploids. Lastly, Berger[13] has presented evidence, adduced from his studies of multiple chromosome complexes of Culicinae, which he believes further emphasizes the insecurity of Darlington's hypothesis of pairing.

Quite possibly the essential condition prerequisite to chromosome synapsis is a high degree of uncoiling of the homologs.[10] Such a degree of uncoiling is apparently the common property of all first meiocytes in which synaptic phenomena occur, and presumably the unique property of the chromosomes of larval Diptera among nuclei of somatic generations (Geitler[22]).

Darlington (especially 1932, 1937) has accumulated an overwhelming amount of data demonstrating that in meiosis the pairing chromosomes associate by twos. It has been abundantly demonstrated that in polyploids the two by two association in synapsis holds. Thus, in a trivalent, one thread synapses for a distance with a second but further along its length may synapse with the third homolog, and for no extensive region do the three threads undergo triple synapsis. Rather than conclude from such data that the associations of chromosomes are by pairs, and that pairing (synapsis) occurs *because* the leptotene threads are unsplit, it certainly seems advisable not to lose sight of an alternative interpretation involving the organization of the chromosomes at meiosis. Inasmuch as polytene chromosomes undergo total synapsis in both diploid and triploid somatic cells despite the number of their component chromonemata, it seems not improbable that at meiosis, regardless of the number (possibly four) of the chromonemata of the leptotene threads, the synapsing chromosomes are bilateral in organization, i.e., constructed in such a manner that each chromosome possesses but one, limited, pairing surface.[24] The polytene chromosome, on the other hand, may be considered radially symmetrical with respect to its synaptic surfaces.

Concluding Remarks.—In the case of the multiple chromosome complexes of the Culicinae, most recently studied by Berger[13,14], it would seem that only the initial stages (viz., polyploidy) towards the formation of polytene chromosomes are undergone. As the chromosome threads do not undertake marked growth in length, it is not surprising that they give no evidence of achromatic banding. Furthermore, it appears not unlikely that the increase in strand number effects an uncoiling of the relic coils of the telophase chromosomes, and gives rise to a bundle of somatically paired, parallel, chromonemata. With onset of prophase, condensation of the chromonemata from the bundles of straightened chromosome threads would result in a bunched association of free homologs (i.e., multiple chromosome complexes) similar to those described by Berger.

Bauer[11] has recently shown that the nuclei of the nurse cells of *Lucilia*, *Pollenia* and *Musca* become polyploid concomitant with pronounced nuclear growth. Polyploidy of these nurse cells is expressed by the formation of an haploid number of banded, polytene-like chromosomes. Bauer holds that these giant chromosomes later dissociate into many small chromosomes, apparently by condensation (helicization) of the chromonemata and consequent separation from the bundle.

Apparently the multiple chromosome complex and Bauer's nurse-cell chromosome represent two different stages in the evolution of the polytene chromosome. In the Culicinae the telophase chromosomes are uncoiled, but, in the absence of marked longitudinal growth, no polytene chromosome is produced. In Bauer's case the chromosomes similarly are uncoiled, but in addition they have also undergone marked longitudinal growth. Identity with the polytene chromosome is not attained by the nurse-cell chromosome for condensation of its component chromonemata sets in before full polytene differentiation has taken place.

The question naturally occurs why polytene chromosomes do not arise elsewhere than in Diptera when nuclear growth occurs with chromosome multiplication but without concomitant mitotic activity. Carnoy (1884), it is true, has recorded or figured polytene-like chromosomes in certain nuclei of Coleoptera, Hymenoptera, Neuroptera, Odonata and the pedal ganglion of *Arion*, but his observations have failed to receive confirmation by recent workers. One factor that seems generally absent outside of the Diptera is the tendency towards somatic pairing (Geitler, 1938). Not only this, but it is suggested that the block to mitotic division in cases of the *Gerris* type (Geitler, 1937, 1938) may occur at a stage after prophasic coiling has set in. For example, the block may occur at premetaphase and the chromatids merely separate. Or, on the other hand, the same end would be attained by a block prior to prophase as in the case of the Culicinae. Thus the nucleus would remain intact as it internally ascends the scale of polyploidy, but would not give rise to polytene chromosomes.

In conclusion I wish to extend my sincere thanks to Professor Franz Schrader, Mr. Arthur Steinberg and especially to Dr. John B. Buck, for much helpful advice and criticism.

[1] Darlington (1937) has employed this useful designation which is adopted in this paper. Koller (1935) coined the term, but stated that he did not propose to use it, preferring "multiple threads." The expression "salivary chromosome" has little to commend it.

[2] Consult the bibliography of Geitler's "Chromosomenbau," *Protoplasma Monographien*, 14 (1938), for references given by date.

[3] Wigglesworth (*Insect Physiology*, 1934) cites Pérez[4] as authority that "even in the extreme case of Diptera, many larval organs (Malpighian tubes; certain muscle groups) are remodelled [during metamorphosis] without much change to form those of the adult."

This suggests that polytene nuclei may be perpetuated into imaginal life in the cases of certain Diptera, viz., *Calliphora;* see also Bauer.[11]

[4] Berger;[13] Bogojawlensky, *Zeit. Zellforsch.*, **22**, 47 (1934); Pérez, *Arch. Zool. Exp. Gén.*, 5 Sér., **4**, 1 (1910); Poulson, *Exp. Génétique*, **3**, 1 (1937); and Trager, *Jour. Exp. Zoöl.*, **76**, 467 (1937).

[5] In that the haploid number of chromosomes or chromonemata bundles are potentially capable of giving rise to a polyploid number of chromosomes (as in Bauer's[11] case), the polytene nuclei are polyploid.

[6] The type of chromosome growth as must be conceived by Metz and his supporters will have the same general effects as here argued.

[7] Somatic pairing and somatic synapsis in part may be considered as different degrees of expression of the same phenomenon. Buck, Painter, Painter and Griffen, Dobjhansky and Tan, *et alii*, have drawn similar distinctions.

[8] Metz[19] seems among the first who have pointed this out.

[9] These considerations lead one to suspect that in Diptera the degree of somatic pairing at metaphase may indeed be dependent upon the extent of effective uncoiling at the preceding prophase. Note especially the observations of Metz[19,20] that somatic pairing at metaphase is in turn due to an intimate association in the prophase when chromosomes are drawn out into long threads. Intensity of somatic pairing, Metz[20] found, decreased from early prophase to metaphase.

[10] Nebel and Ruttle (1937) have suggested that extensive elongation of the first meiocyte prophase chromosomes is an important condition prerequisite to synapsis of homologs. Beasely[12] holds that an increase in nuclear size and a lengthening of the duration of prophase are important factors in meiosis, as they allow complete uncoiling of the chromosomes.

[11] Bauer, H., *Naturwiss.*, **26**, 77 (1938).

[12] Beasely, J. O., *Bot. Gaz.*, **99**, 865 (1938).

[13] Berger, C. A., *Carnegie Contr. Embr.*, **167**, 211 (1938).

[14] Berger, C. A., *Nature*, **141**, 834 (1938).

[15] Berrill, N. J., and Huskins, C. L., *Amer. Nat.*, **70**, 257 (1936).

[16] Bridges, C. B., *Jour. Hered.*, **29**, 11 (1938).

[17] Frolowa, S. L., *Nature*, **141**, 1014 (1938).

[18] Marshak, A., *Amer. Nat.*, **70**, 181 (1936).

[19] Metz, C. W., *Jour. Exp. Zoöl.*, **21**, 213 (1916).

[20] Metz, C. W., *Biol. Bull.*, **43**, 369 (1922).

[21] Metz, C. W., and Lawrence, E. G., *Quart. Rev. Biol.*, **12**, 135 (1937).

[22] Geitler, L., *Protoplasma Monographien*, **14**, 1 (1938).

[23] Geitler[22] has similarly criticized Muller's views.

[24] Limitation of the expression of synaptic forces of genes to a single surface at meiosis might be due to an incomplete envelope of insulating or damping materials about the genes.