COLD SPRING HARBOR SYMPOSIA
ON QUANTITATIVE BIOLOGY

VOLUME IX

Genes and Chromosomes
Structure and Organization

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FOREWORD

When it was decided that this year's Symposium would deal with a problem integrating genetics and the borderline fields of physics, chemistry and mathematics, it seemed logical to select "Genes and Chromosomes—Structure and Organization" as the topic of the symposium. Mathematics is regularly used in the analysis of genetic problems so that whichever topic were selected would have included mathematics. For a number of years, in the studies dealing with the structure of genes and chromosomes physical and chemical methods have been extensively employed, and physical and chemical interpretations have been utilized. At present the work on these problems is at a stage where an organized discussion with participants representing borderline fields seemed profitable.

Since 1936 a small group of biologists, biophysicists and biochemists interested in the gene problem has been holding conferences at regular intervals. This group made up the nucleus of the 1941 Symposium, and the Symposium itself was an expanded gene conference.

In discussions between geneticists and physicists among the questions invariably asked are: What is the approximate thickness of the chromosome threads; when do they divide; how close together do they lie; how tightly are they coiled; and what is their number? The experimental evidence pertaining to these and related questions was presented in the first section of the Symposium dealing with the "Structure of chromosomes as revealed by optical methods." This gave an outline of the known facts which were useful in discussion of problems brought out later.

It is generally assumed that in giant salivary gland chromosomes found in the larvae of flies the primary chromosome thread is multiplied a great many times. The structures which are visible in salivary gland chromosomes are also present in the chromosomes of other cells but they cannot be detected since they are too fine for our microscopes. Thus salivary chromosomes constitute material unusually suitable for studies of fine structures and for the study of changes induced in chromosomes. Known facts dealing with the problems in which salivary chromosomes were utilized were discussed in the second section of the Symposium.

The third section dealt with the "Spontaneous and induced changes in chromosome structure." It is known that chromosomes break spontaneously and also that such breaks may readily be induced by X-rays and similar radiations. Since the occurrence of a break is undoubtedly connected with some chemical reaction, the studies of the breaks may give a clue for an analysis of the chemical properties of chromosomes.

Spontaneous and induced changes in genes were discussed in the fourth section of the Symposium which was designated as "Mutations." An emphasis was placed on the problem of spontaneous mutations which has lately been neglected in similar considerations. It is felt that data on spontaneous mutations may help to clarify certain problems dealing with the induced changes and thus may contribute toward better understanding of chemical processes involved in mutational changes. However a full opportunity was afforded for discussion of changes in genes induced by various physical agents, since at present these data constitute the best material for interpreting the physical and the chemical properties of genes and chromosomes.

"Physical aspects and tools" were discussed in the fifth section of the Symposium. New tools like the electron microscope may well prove an important factor in the study of properties of very fine structures such as chromosomes. Thus the information about the electron microscope may accelerate the work in that field. For interpretation of changes induced in genes and chromosomes by radiation, familiarity with certain physical aspects is essential.

It seems very probable that genes are large organic molecules in which protein and nucleic acid are present. Thus a discussion of the prop-

erties of giant molecules and particularly of proteins, nucleic acid and viruses were topics which logically belonged in this Symposium. Attention of the group was called to the evidence which indicates that frequent atomic interchanges occur in living organic molecules since this may have an important bearing on the visualization of the activity of a gene. A general résumé of ideas brought out in the Symposium was presented as a concluding lecture.

Previous Symposia lasted for five weeks and the majority of participants remained in residence at the Laboratory for a part of that time. This year the program containing approximately the same number of papers was condensed into two weeks. Such modification helped a great deal in keeping the group together and the majority of participants remained in residence during the whole session of the Symposium.

It has been generally assumed that in order that a symposium be a success the attendance should be limited to a relatively small group, since it is believed that a large group hampers the free discussion which is an essential part of a symposium. It has been the policy of the Laboratory to have the Symposia open to all who desire to attend, and this policy was followed this year. I was rather disturbed when instead of the expected attendance of 35 to 50 persons, we had an attendance of about 120. However, as soon as the first session was over, it was evident that the large attendance not only did not prevent free discussion, but that it actually stimulated it. Our Symposium this summer has demonstrated that the interest and not the size of the group determines the success of a conference.

Discussions were recorded by Doctor Katherine S. Brehme and the first draft of the manuscript was prepared after consultation with the participants. After the first draft was revised by those taking part in the discussions, the second draft was prepared and circulated for revisions. The final manuscript was prepared from the second revision of the draft. Thus the discussions should give a true picture of the opinion of the group at the time this volume was written. The volume was edited by Doctor Katherine S. Brehme.

M. DEMEREC

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CHROMOSOME CONTINUITY AND INDIVIDUALITY

H. E. WARMKE

In this introductory paper I should like to review briefly the fundamental concepts of cytogenetics and some of the original experimental evidence upon which these rest, before entering into the subject I was asked to discuss, that of external chromosome structure. I should like to start by mentioning some drawings which Hofmeister made from living cells of Tradescantia in 1848, very close to one hundred years ago. These, of course, were among the beginnings of nuclear cytology; I recall them here to remind you that the science of chromosomes is not exactly an infant, although it is very young in contrast to some of the other sciences represented here at the Symposium. Hofmeister, as is shown by his figures, clearly observed that the nucleus of the spore mother cell resolves itself into bodies (which we now call chromosomes) and that these bodies separate in a definite manner so as to take part in the formation of the daughter nuclei. In 1848, of course, the significance of these structures in heredity was not known; the figures were purely descriptive. These early observations of Hofmeister and others on living cells, however, constitute what I should like to include as first among seven fundamental discoveries in cytology, *1)* that *the cell nucleus may resolve itself into microscopically discrete bodies, which we call chromosomes.*

The perfection of histological techniques and the compound microscope in the latter half of the 19th century ushered in the next important steps in cytological advance. With the introduction of refinements, such as the killing and fixing, sectioning, staining, dehydrating, and mounting of tissues, it was possible to observe more minute and delicate structures. During this period the second important discovery in nuclear cytology was made, *2)* that *somatic cell division is accomplished by a process in which the chromosomes split lengthwise and the identical halves are so distributed that each of the two daughter cells receives the same number and kind of chromosomes which the parent cell contained.* It was shown by Flemming and Strasburger in 1882 that the chromatic threads split lengthwise early in cell division. Van Beneden almost immediately followed up this discovery with the observation that the identical halves of these split chromatic threads separate and pass to opposite poles at anaphase, and each is subsequently incorporated in one of the two newly-formed daughter nuclei. The important process of mitosis was thus described and understood.

Improved microscopic technique was also largely responsible for the next fundamental advance in cytological knowledge, *3)* that *sexual reproduction is characterized by the union of the reduced nuclei of male and female gametes to form the primary nucleus of the embryo, which as a consequence of such union has the somatic number of chromosomes.* It had been known for a long time that in some way or other the presence of a male gamete was required to initiate development in the egg. It was chiefly the researches of Hertwig (1875) and Strasburger (1877), however, which showed that the primary nucleus of the embryo results from the union of two nuclei, one from the egg and the other contributed by the sperm. The brilliant researches of Van Beneden (1883, 1887) on the round worm, Ascaris, completed the picture by showing that the chromosome number is reduced to one half in sperm and eggs and that at fertilization the nuclei of the egg and sperm contribute equally to the chromosome constitution of the offspring and restore the somatic number.

The rediscovery of Mendel's laws in 1900 gave new direction and new impetus to cytological investigations. This called forth what we might designate as the beginnings of true cytogenetics. Sutton, DeVries, and others called attention to the fact that the behavior of chromosomes offered a mechanical explanation of Mendel's laws. The fertilized egg and the organism which develops from it have two sets of chromosomes, one of maternal and one of paternal origin; Mendel had shown that a pea plant behaves as if each cell contained two sets of hereditary units. In eggs and sperm, only one set of chromosomes is present; in like manner, gametes were shown to behave as if they contained only one set of hereditary units. It was the beautiful work of Boveri (1909), however, demonstrating *4)* that *chromosomes maintain a physical and genetic continuity through successive cell generations,* which placed the chromosome theory of heredity on a factual basis.

It is difficult or impossible, even today, to identify individual chromosomes throughout a complete division cycle. During the resting stage the identity of chromosomes is lost to view, and the nucleus appears to be filled with a mass of anastomosing strands or fibers. The laws of heredity require a genetic continuity, not only through a complete division cycle, but through the scores of these divisions between the egg and adult, and any structure assumed to be the carrier of the genetic units would therefore have to maintain physical continuity through successive division cycles. Boveri showed that chromosomes reappear at prophase in the same relative positions they occupied at the preceding telophase, in the early cleavage stages of Ascaris; he thus presented strong experimental evidence that even though chromosomes may be lost to view during certain stages, they do maintain physical continuity throughout successive division cycles.

Workers in this early period generally assumed that all the chromosomes in a cell were alike, both morphologically and genetically. New genetic theory, however, held that only two genes of a kind exist in a somatic cell and only one in a gamete; if chromosomes were to be considered the bearers of genes, this meant that no more than two chromosomes in the diploid organism could be alike genetically, regardless of the total chromosome number.

It was Boveri who established another basic chromosome concept when he showed, *5)* that *the chromosomes of a complement differ qualitatively.* It was known that occasionally a sea-urchin egg is fertilized by two sperms, and that when this occurs the first cleavage is multipolar, and the egg generally is divided into four cells. Subsequent cleavage divisions are bipolar and quite normal, but as a rule, development leads to the production of a variety of forms with structural abnormalities, and most of such larvae fail to survive.

Boveri observed that there is an irregular distribution of chromosomes of the multipolar division and that the cells resulting from this abnormal division rarely have the same number of chromosomes. It was not the number of chromosomes or total amount of chromatin that caused abnormal development, however, because eggs containing the haploid, diploid, triploid, and tetraploid number of chromosomes were known to develop essentially normally. Nor was it an unequal division of the cytoplasm that caused abnormal development, for in the quadripolar cases four equal sized cells are formed, exactly as in the normal embryo at the end of second cleavage. Boveri (1902) therefore concluded that "normal development is dependent on the normal combination of chromosomes, and that this can only mean that individual chromosomes must possess different qualities."

This concept has been confirmed in various other ways since Boveri, notably by Blakeslee and his group, who showed that Datura plants with single extra chromosomes ($2n + 1$ types) differ in appearance depending on which one of the twelve chromosomes is the extra one.

The next concept, *6)* that *the hereditary units, or genes, are arranged in constant linear order within the chromosomes* dates back at least to Roux (1883). Genetic data and the elongate nature of the chromosomes, especially during meiotic prophase when small enlargements, the chromomeres, are seen in a linear order along the chromatic threads, made it logical to assume that genes are arranged as beads on a string. The final cytological proof, however, did not come until much later, when the linear correspondence between genetic loci and bands of the salivary gland chromosomes was shown by Painter, Bridges and others.

The last basic concept I should like to bring before you is *7)* that *the hereditary units are linked together in groups equal to the number of chromosome pairs, but exchange of segments of genes may take place between homologous chromosomes by the process of crossing over.* It soon became evident, after genetic experiments increased in number, that Mendel's principle of independent assortment did not hold in all cases, but that certain groups of factors tended to remain in the combinations in which they entered; thus to be linked. It was mainly through the research of Morgan and his associates that it was made clear that those genes located within the same chromosome did not assort independently, but were linked; the number of such linkage groups being equal in number to the haploid chromosome number. Genetic evidence, however, indicated that there was occasional exchange of segments of homologous linkage groups; this was called "crossing over." This should be accompanied by an exchange of segments of homologous chromosomes, but since homologous chromosomes are normally identical in size and shape an exchange of segments could not be detected under the microscope. Recently Stern on Drosophila and Creighton and McClintock on maize, using translocation stocks in which it was possible to distinguish the two members of a pair of homologous chromosomes cytologically, were able to show that when an exchange of segments of linked genes takes place that there is also a corresponding exchange of segments of homologous chromosomes.

I have not attempted to document this brief review adequately (see Wilson, 1925 and Sharp, 1934 for more detailed treatments and references); also the classification of the fundamental concepts has been largely arbitrary: they might have been expanded into more or contracted into fewer principles, but I think most of the important points have been covered. These principles may not be mentioned again, as such, during the course of the entire symposium; nevertheless, they will underlie the whole program—they will be taken for granted for the most part. I thought it might be well here in the very beginning to review and reaffirm these basic concepts before branching out into some of the newer and more specialized fields of investigation, which will occupy the attention of the symposium for the next two weeks.

I should now like to briefly review some of the more recent knowledge of chromosomes—specifically, what we see under the microscope, using modern techniques.

Chromosome Shape: Chromosomes are more or less rod-shaped bodies but change shape widely depending on the phase of the division cycle. Let us start with chromosomes as they appear at metaphase or anaphase of mitosis. At this stage the nuclear membrane has broken down, and the chromosomes lie in the cytoplasm as sausages. The main body of the chromosome at this stage is made up of a ground-substance called the matrix; and twisted or variously coiled within the matrix lie the chromatic threads or chromonemata, which are the effective bearers of heredity. The outer boundary of the

chromosome proper is called the pellicle by some workers. I must not say more about the chromonemata, because these are the subject of the next two Symposium papers; I am supposed to stay outside of the chromosome. Perhaps I should say, however, that the chromonemata may be revealed microscopically by proper techniques, and also that the pellicle and matrix more or less completely disappear during certain stages of division, thus exposing the naked chromatic threads.

Chromosomes at metaphase and anaphase are usually not just straight rods; they ordinarily have two arms, which may or may not be equal in length. At the point of junction of the two arms the chromosome is usually constricted into what is known as the centric or attachment constriction. The centromere or kinetochore is located at this point of constriction, and may be demonstrated by proper techniques in certain species as small, spherical bodies in the chromatic threads. Navashin has figured them in Galtonia, Trankowsky in Crepis and Najas, and Schrader in the amphibian Amphiuma. In most species they cannot be demonstrated cytologically, but are assumed to be present by the behavior of the chromosomes. The centromere is clearly visible in the pachytene stages of corn, as McClintock (1930) and others have shown. Here the centromere appears as a small transparent sphere connecting the chromatic threads of the arms.

Each chromatid normally has a single centromere. If two centromeres are present in a single chromatid as they may be, for example, after crossing over in an inversion, a dicentric is formed. A dicentric usually arranges itself so that the two centromeres go to opposite poles; it thus forms a bridge across the cell at anaphase, which is resolved only by breakage of the thread. If no centromere is present, as is the case with so-called acentric fragments, the chromatid does not align itself properly on the spindle and usually is lost during cell division.

The centromere is an extremely important structure. It is the interaction of the centromere with the centrosome of animal cells or with the spindle poles of plant cells which orients the chromosomes on the metaphase plate. This is shown by the behavior of acentric fragments, which are usually left off the plate at metaphase and lying out in the cytoplasm; while centric fragments and whole chromosomes behave in the usual manner. The centromeres are also of great importance in initiating separation and poleward migration of the chromatids at anaphase. Separation of the chromatids at anaphase begins at the centromere and proceeds, with the centromere leading the way, until separation is complete and until the chromatids reach the poles.

The position of the centromere along the chromatic thread determines the characteristic shape of the chromosome at anaphase. Whether the chromosome is V, J, or I-shaped will depend upon the relative lengths of the arms—whether the centromere is median, submedian, sub-terminal, or possibly terminal. Terminal centromeres are probably rare or absent in nature; they are known in experimental strains of maize (Rhoades, 1940) and probably also in Melandrium.

The recent work of Pollister in correlating loss of centromeres with supernumerary centrosomes is extremely interesting. In certain snails exceptional, nonfunctional sperms are produced; Pollister (1939) has shown that the centromeres of some or all of the chromosomes are missing and that these chromosomes behave as acentrics at meiosis. In the same cells he has observed supernumerary centrosomes, equal in number to the chromosomes without centromeres. This suggests a definite relationship between centromere and centrosome and may lead to some sort of an explanation of the poleward migration of chromosomes.

Chromosome shape is also modified by secondary constrictions. These resemble centric constrictions, but do not have centromeres, and are in addition to the centric or primary constriction. A good example of secondary constriction is found in Vicia, where such constrictions are found in one or two pairs of chromosomes, depending upon the species (Heitz, 1931). It is probably also correct to consider satellites, those small bead-like bodies attached usually to the ends of chromosomes by a thread, as separated from the main body of the chromosome by a secondary constriction.

Secondary constrictions provide distinctive landmarks to identify certain chromosomes, and also play an important role in nucleolus formation. Navashin (1927), Heitz (1931), and others have shown a definite and constant relationship between secondary constrictions, including satellite constrictions, and the formation of nucleoli.

Chromosome ends are also differentiated to some degree, although there is no visible structure. Experimentally broken chromosome ends tend to fuse with other broken ends to form translocations, inversions, etc., or with themselves to produce chromatid bridges; normal chromosome ends do not unite with other ends to form chromosome chains or fuse to form bridges at anaphase, nor can broken ends be made to attach to normal ends.

The chromosome, then, is a differentiated unit, with a centromere connecting two arms of varying lengths and having autonomous ends. We should not come to think of chromosomes as simply segments of chromatin, but rather as highly integrated and differentiated units.

Chromosome Size: It is difficult to get accurate measurements of chromosome dimensions: First, because length and breadth vary with the stage of the division cycle; they are long at prophase and shorter at metaphase, and it is difficult to draw a sharp line between stages. Secondly, because different killing and fixing agents and different conditions of growth cause different degrees of shrinkage or swelling of chromosomes. Thirdly, because chromosome size may be under genic control as has been

shown by Lesley and Frost (1927). These workers found that chromosomes at MI in a certain strain of Matthiola were considerably shorter than in others. Breeding experiments showed that chromosome size in these lines was genetically controlled, short chromosomes behaving as a simple mendelian dominant to long chromosomes. All these sources of variation probably hinge upon the fact that the chromonemata of chromosomes at metaphase are coiled, with the degree of contraction determining the relative length and width of the overall structure.

Nevertheless, averages give a fair idea of the range in size of chromosomes encountered in different species. The chromosomes of the fungi, for example, are generally small; the average size of the spherical chromosomes in Saprolegnia, according to Mäckel (1928) is less than 0.5 microns. In Trillium, on the other hand, the largest chromosome of the complement averages some 30 microns in length by three microns in width in somatic divisions (Warmke, 1937). Most forms have chromosomes intermediate in size. Maize chromosomes average eight to ten microns in length; the longest chromosomes in *Drosophila melanogaster* are about 3.5 microns. Tradescantia chromosomes average about 10 microns; Melandrium two to six microns (Warmke and Blakeslee, 1940); and Datura 1.5 to four microns (Satina, Bergner, and Blakeslee, 1941). There may be a considerable range in size of chromosomes in the same complement. In Drosophila the second and third chromosomes are about 3.5 microns in length, but the fourth is only 0.3 microns. In Yucca there is also a wide range, five pairs are long, about six microns, and 25 pairs small, about one micron (McKelvey and Sax, 1933).

Chromosome Number: The number of chromosomes in a complement, as the size of chromosomes in a complement, is extremely variable in different species, but is normally constant within a species. The lowest chromosome number known and the lowest possible in a sexually reproducing form is $n = 1$, $2n = 2$, which is found in the round worm, *Ascaris megalocephala* univalens (Boveri, 1909). This example is probably not a very good one because there is the complication of a fragmentation of the chromosomes in somatic cells to form a larger number. *Crepis capillaris* and several species of Crocus, however, have a haploid number of three. Chromosome numbers range from these low ones up to several hundreds. In a summary of almost 2500 species of plants, Fernandes (1931) found that the haploid numbers 12, 8, 7, 9, 16, 6, 10, 14 occurred most frequently, and in that order.

Chromosome numbers are ordinarily constant; a given species will have the same chromosome number regardless of where it is found. There is the phenomenon of polyploidy, however, which is especially common among the plants, where two individuals of the same species or more commonly, two closely related species may have chromosome numbers which differ by a simple multiple of the basic number. Thus, one variety or species may have a haploid chromosome number of nine, and closely related forms may have numbers of 18, 27, 36, etc. By means of the alkaloid, colchicine, we are now able to double experimentally chromosome numbers in a wide variety of plants and produce 3n, 4n, 6n, and 8n plants or sectors almost at will.

The Individuality of Chromosomes: Thus each species normally has a constant number of chromosomes, and the individual chromosomes have certain distinctive features such as position of centromere, relative length of arms, and secondary constrictions or satellites which give them characteristic size and shape. Actually, in most of the species best known cytologically it is possible to distinguish each chromosome of the haploid complement from the others on the basis of morphological differences.

In Trillium, for example, the haploid chromosome number is five. It is relatively easy to distinguish the five chromosome types, either in haploid or diploid tissue on the basis of size and shape (Warmke, 1937). There are three V-shaped chromosomes (median or submedian centromeres): a large V, an intermediate V, and a small V. The remaining chromosomes are a J-shaped chromosome, with one arm two or three times the length of the other, and a knobbed chromosome (sub-terminal centromere).

In maize all ten chromosomes are distinct and can be identified at the pollen grain division (McClintock, 1929) on the basis of length and position of centromere. More recently (McClintock, 1933), the chromosomes have been identified at pachytene; here the presence of prominent chromatic knobs on certain chromosomes, in addition to the other criteria, make identification more certain.

Very recently Satina, Bergner, and Blakeslee (1941) have shown that the twelve chromosomes in Datura are all morphologically distinct. Seven of the twelve in this case were found to bear satellites. These workers have been able to identify the extra chromosome in the primary ($2n + 1$ types), and what is even more striking, they have been able to identify the secondaries. The 9·10 chromosome, for example, bears a satellite on the 10 arm. The 9·9 secondary is equal-armed, and bears no satellite on either arm; the other secondary, 10·10, is equal-armed and bears satellites on both arms, as one would expect.

I think that gives a brief picture of the external structure of the chromosome; I shall now turn our patient over to Drs. Nebel and Huskins who will proceed to operate and reveal some of its internal structures.

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DISCUSSION

Demerec: It is generally thought that chromomeres correspond to genetic loci. There is good evidence for this in Drosophila. Does anyone in the audience know the present status of the evidence in plants?

McClintock: In maize, several color mutants may be referred to one chromomere of a mid-prophase meiotic chromosome. The chromomere in that plant may possess more than one gene.

Demerec: Belling thought the chromomeres in lilies corresponded to genes since the number he observed was comparable to that expected for genes.

Huskins: I am interested in the prophase drawing by Flemming, shown by Dr. Warmke, since loose chromosome ends are visible. This is of interest in connection with the erroneous concept of the continuous spireme, which apparently had its inception in a diagram by Flemming of a continuous thread.

Warmke: Of course the paraffin section technique was in use at this time, and I rather think that Flemming's figure was drawn from sectioned material; in that case what appears to be free ends may have been cut ends. The drawing in question was taken from Wilson, 1928, "The Cell in Development and Heredity," page 126, and was labeled "Endosperm of Fritillaria."

Demerec: A question which has been raised in discussions of the physical aspects of chromosomes is, how far can a chromosome be stretched? The secondary constriction in Drosophila may give good material on this point.

Kaufmann: In some Drosophila nuclei, smeared without great pressure, the chromosome may be so stretched that the piece distal to the pronounced secondary constriction in the left limb of the second chromosome may be on one side of the nucleus, the rest of the chromosome on the other side, with the continuum not visible. In other stages of mitosis, or from the evidence obtained from the salivary chromosomes, it seems that a thread of euchromatin connects the dislocated parts. In 2L then, a small piece of chromonema can be stretched across the diameter of the nucleus.

Demerec: What is the factor of stretching?

Kaufmann: Adjacent chromosome regions may be separated by as much as six micra.

Gates: It is possible that increase in chromosome length is a function of uncoiling of the chromosome spiral. Could Dr. Kaufmann's case be explained in this way?

Schrader: It should be pointed out that in the material of Pollister, referred to in Dr. Warmke's paper, the relation between the number of centrioles and akinetic chromosomes is not approximate but very exact.

Wrinch: What evidence is there as to the moment of duplication of the chromonema? Isn't this the time when the chromatin is not visible?

Warmke: This is a question I should prefer to postpone until later in the Symposium. Both Dr. Nebel and Dr. Huskins I am sure will have something to say about this in connection with chromonemata, and there may also be evidence from X-ray breaks in still other papers.

Jakus: What relation might the granule in the kinetochore have to spinning out of the mantle or traction fiber?

Warmke: I am not able to answer this question.

Huskins: It should be realized that the granule and the kinetochore may be separate entities.

Warmke: Yes, that is certain. The primary constriction is a region of the chromosome in which spiralization does not occur. The centromere, where visible, is merely a tiny dark-staining sphere in the chromatic thread.

Schrader: The granule and the rest of the kinetochore are not indistinguishable. For the present the granule does not matter except for pure cytologists. Cytological and physiological study of the chromosomes may show its exact role later.

The first step in separation of the chromatids is not taken at the kinetochore in the forms with which I am familiar, despite the figure of Haney, which Dr. Warmke showed. The kinetochore is not necessary in all cases for separation.

Warmke: The idea that anaphase separation is initiated by splitting of the centromere, of course is chiefly that of Darlington. I realize that there is much evidence against this; namely the demonstration that the centromere in some species at least may already be split in late prophase or metaphase and also the evidence of Carlson on acentric chromatids.

Schrader: Darlington cannot be pinned down to this, since he shows other figures where splitting does not begin at the centromere.

Metz: In some cases, as Allium, the kinetochore may not be split when separation begins.

Warmke: Colchicine treated material supports Dr. Metz' statement; here the centromere region may remain attached while the chromatid ends are widely separated. Generally in normal material, however, it seems that anaphase separation starts in

the centric region and proceeds out the arms, with the ends of the chromatids being the last to part, regardless of whether or not the ends may have appeared to be free earlier.

METZ: I was not discussing anaphase movement but primary splitting. The chromatids may separate at metaphase or during metaphase, before anaphase separation occurs.

SCHRADER: Two steps are involved. The kinetochore undoubtedly leads the way after the first step has occurred.

BERGER: What does the salivary gland chromosome show about the length of the uncoiled chromonema—does it represent the length of the completely uncoiled chromonema of the resting nucleus? I think the salivary gland chromosome is mistakenly called a prophase stage, but is really a resting stage, and so may be completely uncoiled.

METZ: We do not know anything about this. Most people think the salivary gland chromosome is longer than the uncoiled chromonema, but we cannot tell.

BERGER: Since the resting nucleus chromosome is too small to see, the salivary chromosome gives the only observable material on some points.

DEMEREC: The length of the salivary chromosome was discussed several years ago by C. B. Bridges, Astbury, Wrinch and others; the salivary chromosome is about 100 times as long as the metaphase chromosome, and about 10 times longer than could be accounted for by metaphase uncoiling.

SCHULTZ: Is such a comparison legitimate? Since the size of the metaphase chromosomes themselves differs in the different cells of the organism, we do not know what size of the coiled chromonema to use as a base line.

GATES: Manton, working with Osmunda, measured the chromonema at different stages of meiosis and found that apparent changes in length are the result of spiralization and despiralization, from the evidence of actual measurements.

MULLER: This is related to the question of whether the genes lie directly against each other or are discontinuous and separated by other material. If they are not separated, then differences in length of the chromonema must be due to uncoiling of some kind.

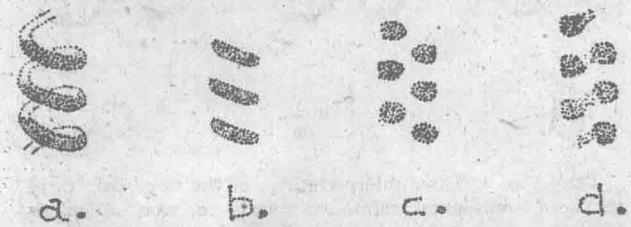
SCHULTZ: This is what I had in mind—the question of the formation of new material in the chromosome, rather than simple uncoiling at telophase.

PIZA: *Tityus bahiensis* Perty, a Scorpion belonging to the family Buthidae, very common in Brazil, has six diploid chromosomes which are provided with two terminal centromeres each. The chromosomes of the spermatogonia are curved or sinuous. At metaphase, because their two ends of chromosomes are in the plane of the equator, their entire body is forced to lie in the same plane. At metaphase of the first meiotic division, the three bivalents, which are rod-shaped, very often form a triangle. The lateral views of these chromosomes show that they are perfectly separated, having the ends turned towards the poles, to which they are connected by spindle fibers. At anaphase the chromosomes of each pair, as they are going to the poles, assume the shape of an arch hanging from the poles by the fibers inserted at its ends. At the second division of the spermatocytes the chromosomes are smaller and thinner, but they show the same general behavior. At anaphase they look like minute parachutes falling down from the equator to the poles. Due to the localization of the centromeres at the extremities, the fragments originating from spontaneous breakages behave like ordinary telomitic chromosomes, and are able to mate with the corresponding parts of the unbroken partner. (Piza; SCIENTIA GENETICA 1:255-261, 1939.)

STRUCTURE OF TRADESCANTIA AND TRILLIUM CHROMOSOMES WITH PARTICULAR EMPHASIS ON NUMBER OF CHROMONEMATA

B. R. NEBEL[1]

In using the microscope on objects of a size ranging close to and below the resolving power of visible light optics, a number of rules must be borne in mind before the images observed or photographed can be translated into three-dimensional models. Two adjacent dark bodies or spots closer than 1/4 of a micron will not be separated. (The theoretically possible slightly higher resolution appears not to be of practical importance in the present material.) A spot or a thread with a diameter less than 1/4 of a micron will not be seen at all or will appear as a very vague shadow. Thus apparent discontinuities of structure may be caused by attenuation of a structure actually continuous. Fictitious images may be caused by refraction and by interference. The human eye distinguishes macroscopically between absorption and refraction by means of experience. At dimensions below one micron this experience must first be built up.

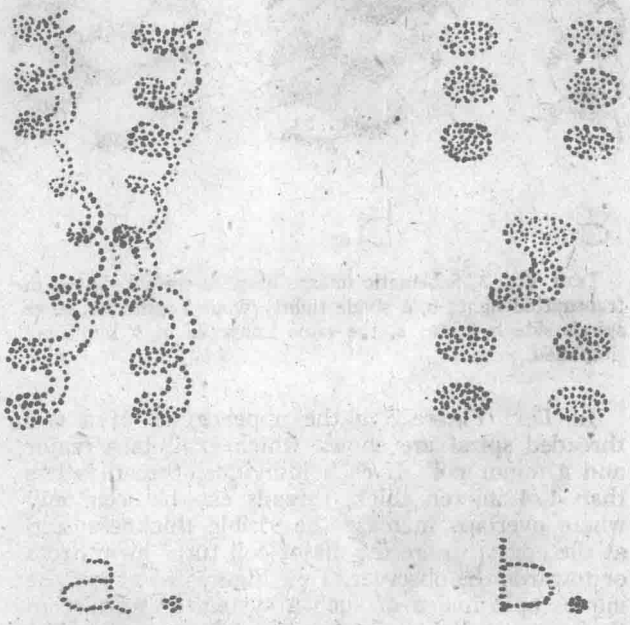


TEXT FIG. 1. Drawing of a simple spiral, diameter of gyres approximately ½ micron; *a*, the object proper; *b*, *c*, and *d* microscopic images of this spiral; *b*, upper focus; *c*, middle focus; *d*, lower focus.

Text Figure 1 shows a spiral and three successive optical sections through this spiral, the gyre of which is more than a micron in diameter and the lumen of which is more than half a micron across. The first two sections, *b* and *c*, conform to expectation but the lowest focus, *d*, does not give a concise image of the lowest level of the gyres. The overlying upper levels of the spiral interfere in such a way as to blur and render indistinct the lowest level.

In Text Figures 1a and 2 the assumption is that the spirals under observation are semi-opaque to the light from the condenser.

In Text Figure 2 it is assumed that the spirals under observation (*a*) are partly transparent, more highly refractive than their surroundings and of a diameter below 1/4 micron. Thus, only those parts of the spiral can be seen where two parts of the thread combine or overlap. Where the spiral is attenuated even two thicknesses will not make it visible. Thus in *b*, showing the assumed image of *a*, the spiral is broken up into discontinuous dots, which upon focusing will follow a short distance but not give a continuous image. The distance between the two turns in the center is below the resolving limit so that the two spirals give only a single image. Just above this spot no image at all is obtained because the individual threads are too thin.



TEXT FIG. 2. Drawing of two spirals, diameter of gyres approximately ¼ micron; *a*, the object proper; *b*, microscopic image of same at all critical foci; details are lost by limit of resolution and through refraction.

Text Figure 3 assumes that a transparent spiral (*a*) is under observation, which is more highly refractive than its surroundings. In *b* three such spirals have been set side by side closely. In *c* the microscopic image of such a system is drawn assuming each spiral to be about 1/2 micron thick. Text Figures 3 *a*, *b* and *c* should be compared also with Figures 7 and 8 which illustrate the seriation of light and dark areas from glass spirals viewed in transmitted light. Text Figure 3 *c* illustrates how

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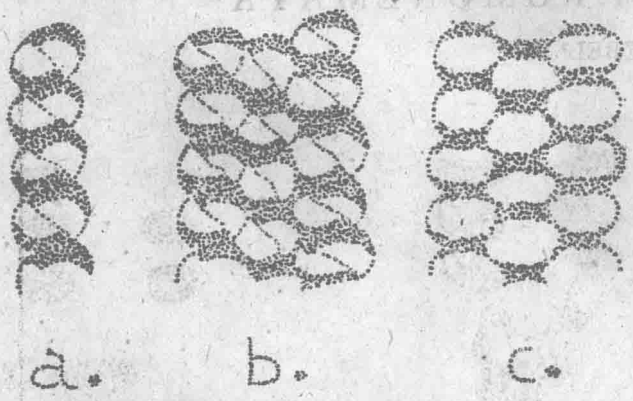
an image of this type will show series of wavy light and dark bands running at a pitch of about 35 degrees across the face of the figure.



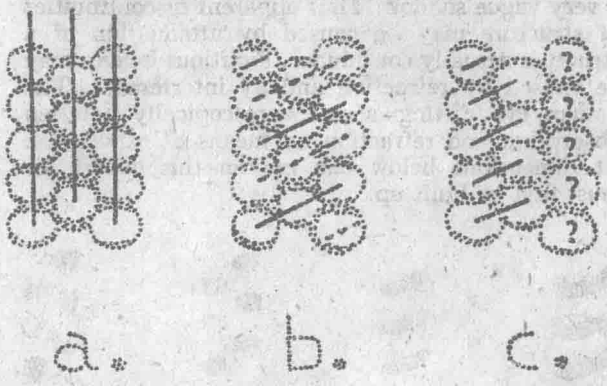
TEXT FIG. 3. Schematic images of glass spirals viewed in transmitted light; *a*, a single tightly wound spiral; *b*, three spirals side by side; *c*, the same image as in *b* but more simplified.

In Text Figure 5 *a* the upper gyres of a two threaded spiral are shown which exhibits a major and a minor coil. If each individual thread is less than 1/4 micron thick, threads can be seen only where overlaps increase the visible thickness, and at the edges, where the major coil turns away from or towards the observer. Text Figure 5 *b* shows the microscopic image of such a system of spirals; in the center a light area is caused by the fact that this structure as a whole gives a "glass rod effect," showing dark margins and a bright central line. The outermost marginal row of black dotted areas on either side corresponds to the turning points of the major coils. The two rows of dotted areas closer to the axis, one on either side, correspond to the first turns of the minor coils, counting inward from the margin on the upper gyres. It is assumed that the entire structure of Text Figure 5 is not thick enough to allow a clear image to be formed of the lower level of the major coils.

Microscopic images corresponding to Text Figures 1, 2, 3 and 5 will be shown in photographs (Plates I and II), in some of which a paired replica has been touched with ink to emphasize the contrast between the light and dark areas of the photograph. On the correct assignment of the photographs to a corresponding schematic spiral type depends the correct interpretation of chromonema number and behavior.

MEIOTIC CHROMONEMATA

Figures 1, 2, 5 and 6 illustrates spiral types which may be assigned to the pattern of Text Figure 1. In Figures 1 and 5 the upper focus of the major spiral of meiosis is shown, transgressing the full



TEXT FIG. 4. Three interpretations of the same pattern in terms of transparent refractive spirals; *a*, axes of spirals vertical, side by side; *b*, axes of spirals diagonal; *c*, if a single gyre of a vertical spiral should give two rows of light spots, the third row "(?)" not accompanied by a fourth is unaccounted for.

FIGURE LEGENDS FOR PLATE I (see facing insert)

FIGS. 1 and 2. *Trillium grandiflorum* first meiotic anaphase chromatids; 1 upper, 2 lower focus, showing tertiary split and minor coil; the lower focus does not give a clear image of the lower level of the gyres; mark the diameter of the minor spiral as shown near arrow in 1.

FIGS. 3A and 3B. Late first anaphase in Trillium; upper focus showing light and dark dots in checker board arrangement in two chromosomes; 3A untouched; 3B the dark areas have been blackened and dotted with india ink.

FIGS. 4A and 4B. Late first anaphase in Trillium in medium focus; 4A individual chronemata appear to give separate images in area indicated by arrow; 4B the same picture touched with india ink to mark apparent position of individual chromonemata in favorable area.

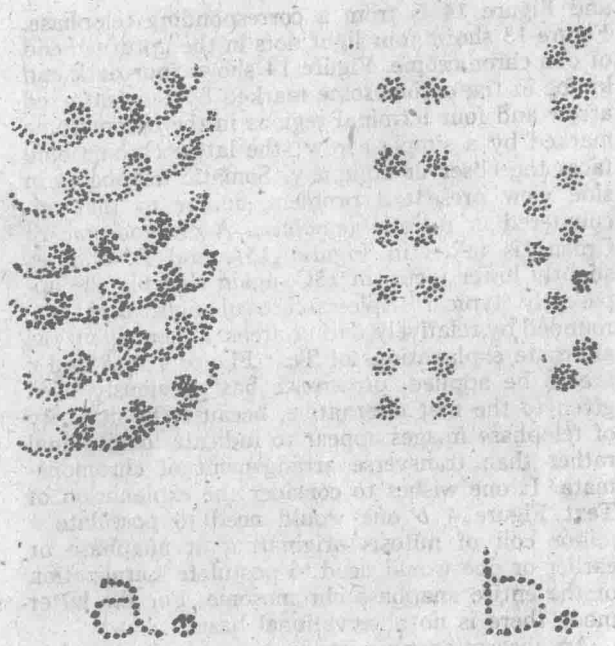
FIGS. 5 and 6. *Tradescantia reflexa* absorption images; 5 prometaphase of meiosis coils stretched with half normal Ringer at pH 7.6 showing minor coil and secondary split; 6 first anaphase showing tertiary split at turning points of gyres and at upper end of left chromatid.

FIGS. 7 and 8. Glass models photographed in transmitted light; 7 single spiral closely coiled; 8 two single spirals side by side; touched with india ink to make dark areas plain; the central row of light spots represents interspaces between the two spiral columns.

FIGS. 9 and 10. Late somatic prophases in Trillium showing four chromonemata at end and transgressing the kinetochore respectively. (Slides loaned by Dr. L. W. Sharp.)

FIGS. 11 and 12. Glass spirals photographed head on in transmitted light; 11 single spiral snugly coiled transmits light to end; 12 two spirals loosely coiled transmit little if any light to ends.

FIGS. 13 and 14. *Tradescantia reflexa*, microspore divisions; 13 metaphase in end view, showing 4 light areas in right hand partner; 14 telophase showing chromosome ends at different angles with reference to the observer, marked by simple and feathered arrows. (Compare figs. 11 and 12 with 13 and 14.)



TEXT FIG. 5. Drawing of the upper level of two stranded spiral with major and minor coil; *a*, physical image; *b*, microscopic image, with central area deleted due to "glass rod effect."

width of the chromosome. In Figures 2 and 6 the lower medium focus of this type of spiral is shown, giving the turning points as dots and the lower level of the gyres somewhat indistinctly.

A casual inspection of Figure 1 (the distal three gyres of the major spiral are under discussion) seems to show a light median line. This suggests two separate vertical columns of two stranded spirals. The lightening of the median line is due to the "glass rod effect" of the entire chromosome. Careful visual observation shows the spiral to transgress the entire width of the chromatid involved.

What is the optically visible thread number in the first reduction division in Tradescantia and Trillium? Figure 5 shows only the two chromatids for a prometaphase chromosome; this photograph also shows the minor coil which will be discussed below. Figure 1 shows two threads in the end of a half dyad at first anaphase in Trillium and Figure 6 shows half a dyad with its mate in first anaphase of Tradescantia. In Figure 6 the doubleness is clear at the end and at several turning points, while in Figure 1 the doubleness is suggested by the contour of the turning points. In Trillium and Tradescantia first meiotic anaphase reveals the presence of half chromatids, that is, the tertiary split is visible, and the dyad is fourpartite.

With further progress of nuclear division, the first telophase in Trillium yields the images shown in Figures 3A and 3B, 4A and 4B. The upper focus no longer shows a continuous band across the width of the chromosome but instead a checkerboard or honeycomb pattern is seen. This type of image may correspond to the Text-Figure 3*b* and *c*. Two spirals with wide gyres have been replaced by four parallel non-coaxial spirals with narrow gyres. This conclusion is made more likely by the lower focus image shown in Figures 4A and 4B which seem to show four more or less parallel irregularly coiled strands running through the length of the chromosome. What appears as four more opaque or thicker regions of the chromonemata has been marked with ink in Figure 4B. If this interpretation is correct the dyad is now eight-partite showing the quarternary split. This interpretation requires that an individual thread contracts in length between anaphase and telophase approximately by a factor of two

FIGURE LEGENDS FOR PLATE II (see facing insert)

FIGS. 15A, 15B, and 15C. Somatic telophase chromosome of Crocus sp. showing the honeycomb pattern at different focal levels; 15A untouched photograph; 15B dark areas of photograph accentuated with ink; 15C the same at different focal level.

FIGS. 16A and 16B. Telophasic somatic chromosome fragment; 16A untouched; 16B dark areas accentuated with ink; four chromonemata in parallel or two chromonemata plectonemically coiled with a minor somatic coil superimposed on the "standard" somatic coil.

FIGS. 17 and 18. Tradescantia microspore division anaphase; 17 at least two spirals are indicated at the points marked by arrows; 18 the same; if two strands are present they may be para- or plectonemic; if only two strands are present they appear to show a minor somatic coil.

FIGS. 19A and 19B. Tradescantia meiosis prometaphase pretreated with ½ normal Ringer pH 7.6; 19A shows checkerboard or ratchet arrangement of dark spots indicating the presence of the minor meiotic coil; 19B dark areas emphasized by spotting with ink. The two strands involved are considered paranemic.

FIGS. 20 and 21. Pachytene and first anaphase of Trillium; 20 the individual pachytene threads appear optically single; spacing between successive dark areas ±¼ micron; 21 anaphase figure juxtaposed to show that here also successive dark areas are approximately ¼ micron apart.

FIGS. 22, 23 and 24. Somatic prophase and telophase of Amblystoma sp. (slide loaned by Dr. C. L. Parmenter) and first meiotic anaphase in Melanoplus sp.; 22, no subdivision of chromatids appears possible; 23, differentiation into chromatids appears obvious; 24, no subdivision of chromatids appears visible; since the visible coils in Melanoplus are about as large as the minor meiotic coils in Tradescantia, coiling in Melanoplus is either relatively more coarse or, if it contains the same details as Tradescantia, these are below the range of optical resolution.

Magnifications

FIGS. 7, 8, 11, 12	x 1
FIGS. 22, 23, 24	x 2000
FIGS. 1, 2, 3, 4, 5, 6, 9, 10, 13, 14, 17, 18	x 3000
FIGS. 15, 16, 19	x 3500
FIG. 20	x 4500
FIG. 21	x 5500

and increases in thickness from below the limit of resolution to above 1/4 micron.

There are two other alternate explanations for the telophase images of Figures 3 and 4. If the minor coil of meiosis expands but slightly from its anaphase diameter and if the thickness of individual threads approximately doubles, then the honeycomb image of Figure 3 may be the result of the expanded minor coil without an increase in thread number. In this case the telophase of the first division in Trillium shows only the tertiary split. The foregoing possibilities of interpreting the first telophase chromatid of Trillium are diagrammatically represented in Text Figures 4*a* and *b*. One has the task of seriating three parallel rows of staggered spiral gyres. Text Figure *a* seriates the spots vertically, *b* seriates the same pattern in terms of a major and a minor coil, adjacent gyres running diagonally across the face of the pattern.

If Figures 3A and B are to be interpreted according to Text Figure 4*b* then Figures 4A and B conform to the scheme laid out in Text Figure 5*a* and *b*. The eight black ink areas entered in Figure 4B correspond to eight dotted areas at any one of the three levels shown in Text Figure 5*b*.

Finally there is a third possibility of seriation, mentioned for completeness rather than for its likelihood (Text Figure 4*c*). Figure 8 shows two glass spirals. In the second gyre from the top, two light spots are visible for a single turn in *each* spiral. According to this phenomenon the "triple ratchet" pattern could be seriated according to Text Figure 4*c*. This leaves one row of spots unaccounted for. There is another objection to this last interpretation. In Figure 8 the dark bridges which separate adjacent white spots of the same gyre are too narrow to promise optical resolution in terms of chromosomal dimensions.

Somatic Chromonemata

Figures 9 and 10 are taken from Trillium slides loaned by Dr. L. W. Sharp. They are taken late in prophase and there can be no doubt that four strands in Figure 9 transgress the kinetochore or form part of the kinetic region in Figure 10.

If one wishes to investigate how far back the fourpartite condition extends in the somatic cycle it becomes necessary to use more indirect means of investigation. Previous studies have led to the conclusion that somatic telophase shows four chromonemata in Trillium and Tradescantia. This finding requires recapitulation. Chromosome ends have been used extensively to show that somatic chromosomes at telophase appear to contain four chromonemata. For comparison glass spirals were photographed in end view and Figure 11 shows that a snugly coiled spiral will transmit light whereas a loosely coiled one (two are shown in Figure 12) will not.

Figures 13 and 14 may be compared with Figures 11 and 12. Figure 13 is from a late metaphase in Tradescantia in the first division in the pollen grain, and Figure 14 is from a corresponding telophase. Figure 13 shows four light dots in the upturned end of one chromosome. Figure 14 shows four dark end knobs in the chromosome marked by the feathered arrow and four terminal regions in the chromosome marked by a simple arrow; the latter chromosome faces the observer obliquely. Somatic telophases in side view present a problem similar to that encountered in meiotic telophase. A chromosome of Crocus is taken in Figures 15A and B, and, in slightly lower focus, in 15C, again showing the apparently typical triple-ratchet of light dots surrounded by relatively darker areas. If here again the alternate explanations of Text Figure 4 *a*, *b* and *c* are to be applied, preference has previously been given to the first alternative, because the majority of telophase images appear to indicate longitudinal rather than transverse arrangement of chromonemata. If one wishes to consider the explanation of Text Figure 4 *b* one would need to postulate a minor coil of mitosis originating at anaphase or earlier or one would need to postulate spiralization of the entire anaphase chromosome. For the latter mode there is no observational basis.

An absorption type image was recently found in Tradescantia pollen grain material from 75 r X-radiation. Figure 16 A and B show this telophase fragment which contains four chromomeric regions side by side in its lower half. Of these three are plainly visible in a single photograph with the fourth one partly showing under the first chromomeres on the side of the arrow. The upper end of this fragment also appears fourparted. It thus seems that the telophase chromosome in somatic divisions of Tradescantia at least may be fourpartite.

Figures 17 and 18 show early anaphase in side view. The regions marked by arrows in Figure 17 indicate the presence of two chromonemata, corresponding in part at least to the conditions illustrated in Text Figures 2 *a* and *b*.

The "V"-shaped chromosome of Figure 18 shows a "triple-ratchet" distally in the long arm and a "double-ratchet" proximally. The chromosome marked with the three-feathered arrow is pictured only in extreme upper focus. It shows a "double-ratchet" in this focus and probably corresponds closely to its mate.

The photographs so far available of somatic anaphase chromosomes present extreme difficulties of interpretation. Simple cases, where straight bands transgress the width of the chromosome are the exception. The double and the "triple-ratchet" can be explained in two ways, according to Text Figures 1 *c* and 4 *a*, or 4 *b*. The structure of the somatic anaphase chromosome in Tradescantia is thus still considered a puzzle. Certain images, such as Figure 16, suggest four chromonemata non-coaxially spiraled in paranemic arrangement. It is possible that early anaphase figures as shown in Figures 17 and 18 represent two paranemic coils engaged simultaneously in separating from each other and in