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CHROMOSOME STRUCTURE

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INTRODUCTION

The present discussion on certain phases of the microscopic structure or chromosomes does not attempt completeness, nor is it intended to be utterly impersonal. There is a series of recent reviews available, none of which is without merits. The authors are as follows: Bauer (1937*a, b;* 1938), Darlington (1937*a;* 1939*b*), Frey-Wyssling (1938), Gates (1938), Geitler (1938*a*), Heberer (1938*a*), Heitz (1938), Marquardt (1937*a;* 1938) and Straub (1938*b*).

Preference is given in the present paper to recent work on plants. However, animal chromosomes are not omitted wherever the work is considered of general importance.

Terminology will be defined so as to avoid misunderstanding, and it is hoped that the committee on nomenclature will soon eliminate unnecessary synonyms.

TERMINOLOGY

kinetochore = spindle fiber attachment region = centromere.

akinetic = acentric = without a kinetochore.

dikinetic = dicentric = with two kinetochores.

chromonema = an optically single thread within the chromosome, a purely descriptive term without functional implications.

chromatid = the longitudinal *half* of an anaphase, interphase or prophase chromosome at mitosis. During meiosis chromatid is used to signify one of the four strands involved in crossing-over and visible after pachytene. At metaphase of the second reduction division the chromatid becomes a chromosome again, so that from then on the term is used as in mitosis. During first meiotic ana-

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phase a chromosome has two chromatids; during second meiotic anaphase again two, but the entire chromosome of the second anaphase may be referred to as "a chromatid of meiosis."

nemamere = one of the physical units composing the gene-string or genonema. The genonema may carry the genes, but not every gene may be a definable physical unit. Thus a nemamere may be composed of several genes, or a single gene may extend over several nemameres. The nemamere is of molecular or of micellar size and hence not visible. It governs the biophysical reactions of the gene-string.

tetrad = geminus = bivalent of first meiotic division.

quartet = a group of four microspores, not to be called tetrad.

diplochromosome = two identical chromosomes held together at the kinetochore and originated by doubling of chromosomes without separation of daughters.

matrix = kalymma = hyalonema = ground substance, surrounding the chromonemata; usually less chromatic than these, making up the body of the chromosome; by some believed to form a pellicle or sac on the surface of chromosomes or chromatids, by others believed to be more or less completely apportioned to definite chromonemata.

THE NUMBER OF INDIVIDUAL THREADS CONTAINED IN A LARGE SOMATIC PLANT CHROMOSOME

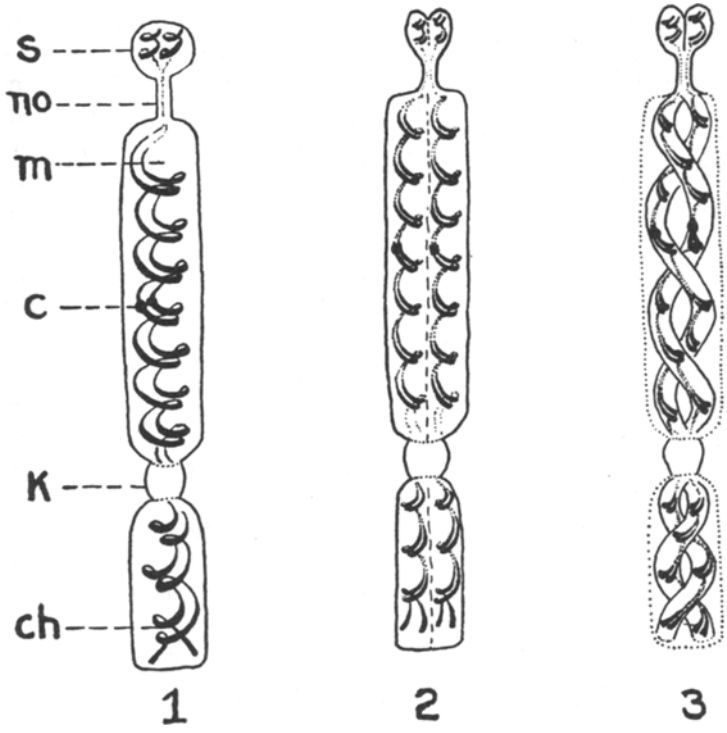
To draw a diagram of a chromosome may not now be done with finality since workers are at present not unanimous in their interpretation of details. Diagrams 1 to 3 thus represent the writers' viewpoint (*cf.* Nebel and Ruttle, 1936; Ruttle and Nebel, 1937; Nebel, 1937*a, b;* Nebel and Ruttle, 1937).

Diagrams 1 to 3 illustrate a somatic anaphase chromosome. The chromosome has a long and a short arm, the former carrying a satellite *s*. The satellite is connected with the chromosome by a neck region *no*. The kinetochore *k* is drawn as a single body. The chromonemata *ch* are drawn as though they were wires or solid threads. They carry occasional chromomeres *c*, usually not seen in this stage, and are surrounded by a homogeneous substance *m*, the matrix.

In diagrams 1, 2 and 3 chromonemata are shown closely appressed so that they may be mistaken for one.

In diagram 2 the chromosome is divided into two chromatids, and

each chromatid contains a closely appressed pair of chromonemata or perhaps a single chromonema, the cross section of which would represent a dumbbell.



Figs. 1, 2 and 3: Three different aspects of the same chromosome. *s*: satellite; *no*: neck region; *m*: matrix; *c*: chromomere; *k*: kinetochore; *ch*: chromonema; three aspects of what is believed the same fundamental structure. In Fig. 1 only two mutually appressed threads can be resolved, in Fig. 2 four threads are arranged in two separate columns (chromatids). In Fig. 3 the chromatids are relationally coiled dextrorsely in the short and sinistrorsely in the long arm. (See text p. 564.)

In diagram 3 the basic structure is the same as in diagram 2 except that the chromatids are wound around one another in what is known as a relational coil. Also it would seem that there must be two matrices, one surrounding the chromatids and another constituting the ground substance of each chromatid. The three diagrams may be looked upon as successive stages of the same anaphase chromo-

some or as alternative images of a general pattern which may express itself only in one of many ways. Thus in certain material the anaphase chromosomes may show only the aspect of diagram 1. In other material the aspect of diagram 3 may prevail, but the internal structure of the chromatids may be obscured. Thus it is not feasible to call any one of the three diagrams generally typical.

The question as to how many chromonemata may actually be seen in large somatic plant or animal chromosomes has been summarized by Sharp (1934), later by Kaufmann (1936) and still later by Geitler (1938*a*). Darlington (1937*a*) still maintains that the chromosome does not split until the division commences during which half-chromosomes separate. "If there were a structure in a cell body which had no effect on its behavior its investigation would be of very little interest" (Darlington, 1939*a*). This statement, although made in another connection, will scarcely be subscribed to by many colleagues, as investigation is not committed to track down known processes and to overlook facts which have no immediate place within the realm of "known" effects. Perhaps, however, the statement furnishes the reason for Darlington's refusal to consider anaphase chromosomes as split.

There are in the main three schools of thought, the first of which, just mentioned, believes the leptotene thread and correspondingly somatic interphase chromosomes to be single. Recently, Afify (1938), Gustafsson (1936), Mather (1937*b*), Overijns (1938) and White (1937) have adhered to this interpretation.

The disagreement on thread number will probably appear somewhat artificial 20 years hence when more knowledge will be available to understand why the same element may change its appearance confusingly. The one-thread-school at present perhaps overemphasizes genetical function, cell mechanics of mitosis and meiosis and x-ray reactions, all as they are understood and interpreted today.

The majority of workers today still consider the somatic chromosome bipartite from anaphase to prophase and four-partite from then until metaphase. The leptotene logically is then bipartite. Recent publications from this group include Atwood (1937), Creighton (1938), Gates (1938), Geitler (1938*a*), Huskins and Smith (1934), Jeffrey (1937*b*), Koshy (1937), Levan (1939*a, b, c*), Naithani (1937), Sax (1938),* and Wolcott (1937; 1939). Of

* Sax, 1939, Proc. Nat. Acad. Sci. 25: 225–233, considers the interphase chromosome single.

these Gates writes: "Whether these (the somatic anaphase chromatids) are possibly in turn composed of split halves may be left to the future; the present evidence for this view is unconvincing."

However, a growing group of investigators has at times seen the chromatid as a bipartite structure or as if it were split. Knowing that any visible chromonema may contain concealed within it grossly from 20 to 100 gene-strings, and if such individualized gene-strings actually exist, and in view of the recent knowledge of polytene chromosomes, there is no *a priori* reason to consider the existence and visibility of smaller subunits within the chromatids as impossible. Extreme care with optical interpretation is, however, required. The split within chromatids may be observable in one part of the fixed chromosome and not in another. Upon stretching, it becomes obscured, and its demonstration requires reagents which will contract and swell the chromatic elements, rather than treatments which will expand and attenuate them. It is not claimed that the half-chromatids are not vital artefacts and may not be the result of or become accentuated by abnormal conditions. In either case the presence of half-chromatids in fixed material is believed to give indication of the bilateral structure of the chromatid, and if it is a vital artefact it gives indication of what *in vivo* may be preformed on a somewhat smaller scale or in a somewhat less progressed state of differentiation.

Straub (1938*b*), without giving a specific reason, considers the work of Haga (1937) critical proof of the existence of half-chromatids in microscopic dimensions. Likewise, Kuwada (1938) has now observed the "tertiary split"—an unfortunate term for half-chromatids—in meiosis of *Tradescantia,* pictured by Sharp in his text of 1934. Carlson (1938*a, b*) has produced evidence of half-chromatid lesions in *Chortophaga viridifasciata* (1938*b,* fig. 18A), although he himself considers half-chromatids not definitely established. Levan (1939*b*) sees half-chromatids after colchicine treatment in a stage corresponding to second meiotic telophase. However, he believes this to be "unreal" because on stretching of the chromonemata they become single. Would it not be equally justified to call the singleness artificial? Heberer (1938*a, b*) has shown half-chromatids in optical cross sections in Copepoda. McClintock (1938*a*) considers the existence of half-chromatids in corn meiosis as a possibility. Nebel (1937*a, b*) considers the half-chromatid a general morphological characteristic of large chromosomes. Per-

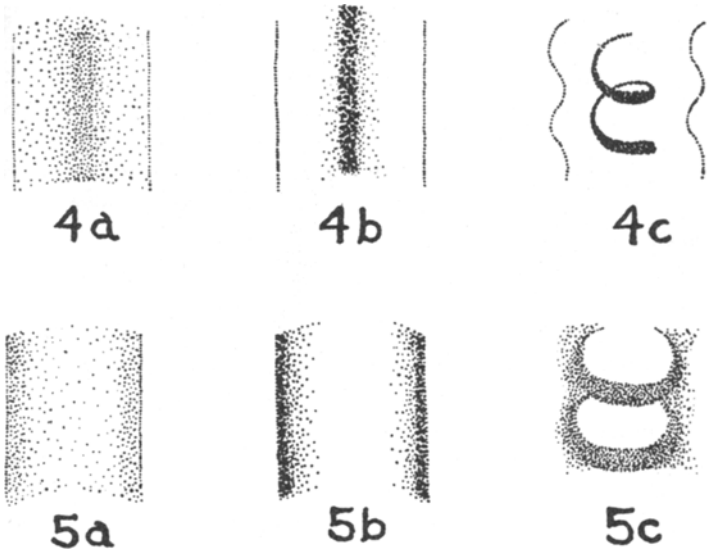
haps proponents and opponents of the half-chromatid viewpoint have both overstressed their positions. Proponents should generally admit that the half-chromatid may be a vital artefact and may be more clearly visible under a distinct set of circumstances. Opponents should not be overly intolerant even if the majority of figures which they observe do not obviously show half-chromatids.

Geitler (1938*a*) has tried very hard to give the half-chromatid interpretation a fair consideration. As counter evidence he gives great weight to an experiment by Wada (1937) in which living chromosomes were stretched and in which it was shown that they consisted of a much compressed single spiral of which the gyres touch. Wada's evidence is of no import in this connection since it is universally true that where multiple threads of chromatin are stretched they will, if closely adjacent, coalesce to form a single thread. Leptotene is an example of this phenomenon. Geitler further believes that any image which does not show an axial thread within a clear matrix should be discarded.

Thus the most cogent evidence is arbitrarily dismissed. A clear matrix with an axial thread resembles an artefact produced under circumstances opposite to those which make half-chromatids visible. Such an image is produced by partial autolysis leading to an axial coagulation of any preexisting multiple number of threads. On the other hand, a treatment which will slightly shorten and swell individual threads after separating them will show half-chromatids or at least their refraction patterns. Unfortunately, there is no method available which will condense—rather than swell—chromonemata and at the same time separate them. By swelling and separating them one loses the "light-absorption" image of a "wire in test tube." After having made a preparation with swollen and separated chromonemata, one must be ready to interpret it and not discard it as Geitler would. Geitler (1938*a*) refers to Koerperich as having drawn the most perfect pictures of telophasic refraction patterns—at telophase it is generally impossible to obtain a "wire in test tube" image. For this reason many desist from interpreting at this point. Yet the telophase images may be readily seriated and interpreted.

To do this a distinct group of colloidal changes must be taken into account. With Alexander (1937) they should be called pectization (Entquellung) and peptization (Quellung). In diagram 4*a* a piece of a chromonema is shown in the pectized state. In diagram

5*a* the corresponding peptized state is shown. In diagrams 4*b* and 5*b* the respective fixation images of such a piece of the chromonema are shown. Diagram 4*b* corresponds to the axial thread image; diagram 5*b* corresponds to the image of a glass rod. In diagrams 4*c* and 5*c*, respectively, the pectized and peptized conditions are applied to a coiled thread. These constructions may now be applied to figures taken from observations of different investigators.



Figs. 4 and 5: Fig. 4*a*: A piece of a chromonema in the pectized state. The density appears to increase centripetally. Fig. 5*a*: A piece of a chromonema in the peptized state. The density appears to increase toward the margins. Figs. 4*b* and 5*b*: The same pieces after fixation. Figs. 4*c* and 5*c*: The same or somewhat longer pieces coiled into two gyres of a helix. (See text p. 568.)

The changes and type images in an anaphasic chromosome between somatic metaphase and telophase may develop as follows: two coils apparently run parallel; the matrix is weakly stained (diagram 6*b* after Sax and Humphrey, 1934, Bot. Gaz. **96**: 353–362, Plate IV, fig. 15). The obvious thread number is 1, which upon closer inspection appears split, so that careful observers speak of two threads. This figure upon peptization yields an image corresponding to diagram 6*a*. The next stage is anaphase which may be typified by fig. 2 of Sax and Sax (Jour. Arn. Arb. **16**: 423–439.

1935. plate 162). The lower half of the figure shows anaphase in *Vicia faba*. The coils are indicated by refraction patterns, not merely by light absorption. These patterns are schematically drawn in diagram *7a*. They represent the swollen condition of two threads as drawn in diagram *7b* when in the pectized state. Coming finally to telophase, the figure of Koerperich as shown on page 80 of Geitler (1938*a*) may be used (diagram 8*a*) which represents the same general condition as at anaphase, except that another pair of spirals has become visible so that the total number observable in side view of telophase is three. The light areas of the *"a"* diagrams correspond to peptized threads; the dark are the interstices, while in the *"b"* diagrams the pectized threads are dark.* This interpretation is confirmed by the fact that under exceptional conditions one may see four threads in each prospective anaphase chromosome at metaphase, and end views of such chromosomes present an image as shown schematically in diagram 9. In summary: The four-thread viewpoint should under all circumstances be prefaced by the necessary reservations. However, potentially in animals and plants with large chromosomes these are considered four-partite from anaphase to prophase, when they become eight-partite. Hence the leptotene thread is actually, although often not visibly, four-partite, and another split occurs during meiotic prometaphase making the metaphase chromosome of the first meiotic division potentially eight-partite and the whole tetrad 16-partite (best material: *Trillium*). During the second meiotic metaphase no further split occurs so that the anaphase chromosome of the second division is again four-partite. In many cases the splitting does not become obvious until telophase, and wherever stretching of chromonemata follows splitting a split previously established may become closed again, as in the early stages of somatic prophase during leptotene of meiosis, and in late interkinesis wherever a nuclear stage of interkinesis between first and second reduction division occurs (*cf.* Nebel and Ruttle, 1936). This reuniting of chromatic threads has been insufficiently emphasized.

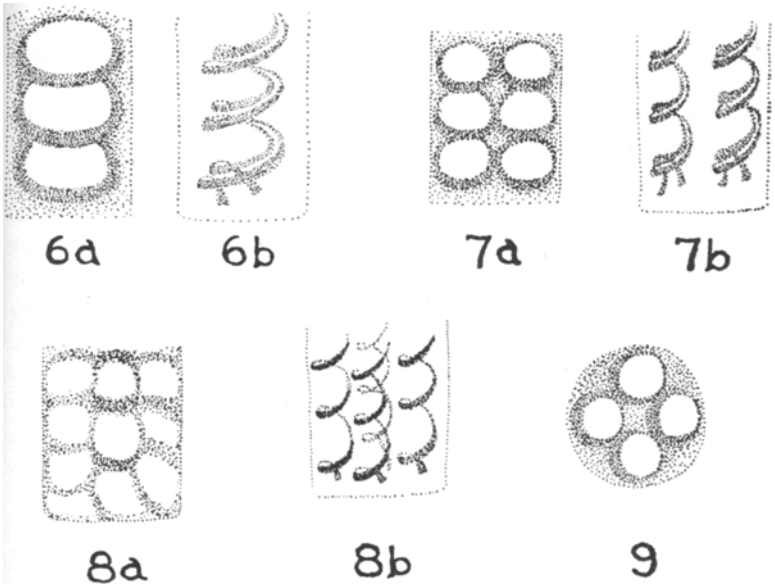
THE EFFECT OF X-RAYS ON CHROMOSOMES

Recent work on the effect of x-rays on chromosomes has continued to show the complexity of the problem and has opened the

* In figs. 6 and 7 pairs of threads give a single refraction column; in fig. 8 each thread gives its own column, due to orientation.

subject for further research. Several subtopics may be distinguished.

Chromosomes appear to be endowed with a natural tendency of reconstruction. Radiation affords an opportunity for this mechanism to exert itself. Radiation effects are thus recorded as results of the component effect and response, and the boundaries between effect and response are vague.



Figs. 6*a* to 8*b*: Diagrammatic illustrations of microscopic images of multiple threads in the peptized (*a*) and in the pectized (*b*) state. In Figs. 6*a* and *b* the images of two appressed threads is shown. In Figs. 7*a* and *b* the images of 4 threads appressed into two pairs are shown. In Figs. 8*a* and *b* the images of four independent threads are shown. Fig. 8*a* is redrawn from Geitler after Koerperich. Fig. 7*a* corresponds to a photograph by Sax and Sax. Fig. 6*b* corresponds to a figure of Sax and Humphrey. Fig. 9 is an optical section or end view of a chromosome piece as shown in side view in Figs. 8*a* and *b*. (See text p. 569.)

There exists a difference in the reaction of the chromosome depending upon the nuclear stage during which radiation is applied; these differences are qualitative and quantitative. Primary, atomic and molecular, effects of radiation under various conditions of nuclear stage must *a priori* be very similar; hence cytogenetic problems of radiation effects may be called response problems, whereas the primary effect is at present under investigation by physicists and chemists.

Marshak (1937; 1938*a*, *b*; 1939) found that ammonium hydroxide in concentrations of $10^{-8} \times 1$ to 8 decreased the natural prophasic sensitivity of chromosomes. His working hypothesis postulates that the action of electrons on the positively charged surfaces of dividing chromonemata constitutes the primary x-ray effect. The counter-experiment, namely, treatment of cells with atmospheres of CO_2 up to 80 per cent, did not increase the sensitivity of the chromosomes. However, the hypothesis has considerable appeal. The effect observed under ammonia was probably brought about by removal of sensitive prophases from the material to be rayed. It is well known that under adverse conditions prophases will revert to interphases. Whether surface charges are the deciding mechanism in the phenomenon observed remains to be proved.

The primary x-ray effect is upon atomic nuclei. Hence Marshak's observation constitutes a change in the response mechanism of the living material, not a change in the primary effect. Perhaps one can visualize the process as follows: During all stages except prophase genes are longitudinally tied together by nucleic acid molecules straddling from one gene to the next and presenting cross ties by means of salt linkages (Frey-Wyssling, 1938). During prophase these cross ties are loosened; ammonia treatment causes them to be reestablished by intercepting the nucleic acid synthesis and stabilizing a system otherwise at that time labile. Unpublished results of Nebel and Helwig, using CO_2 atmospheres on grasshoppers in connection with x-rays, confirm Marshak's observation that one can not sensitize nuclei to radiation by keeping the material in a CO_2 atmosphere. Gustafsson (1936) obtained increased sensitivity to radiation under increased water content of his material, but again the effect was not a primary one; soaking of the seeds brought more nuclei into the sensitive prophase stage.

Sax (1938) systematically classified qualitative differences in x-ray response according to the nuclear stage at the time of treatment. A few hours after treatment metaphase chromosomes show no breaks but merely clumping and sticking-matrical effects. This is confirmed by Marquardt (1938) and others (see Goodspeed and Uber, 1939). During the first 24 hours all chromosome breaks are single, *i.e.*, not translocations. Corresponding ends of broken sister chromatids fuse, thus leading to a bridge and a fragment at the next division. After the second day single breaks are rare and there are no fusions between sister chromatid ends. After 48

hours chromatid lesions become rare. Up to that time they are in the majority. No temperature effect is found, indicating that the response reactions occur so rapidly that a slight change in rate can not be detected. Sax also finds a marked decrease in sensitivity in interphase extending possibly through the preceding metaphase. Perhaps this low sensitivity is explainable through a high rate of repair. The prophasic sensitivity is laid to the existence of torsional stresses. Aberrations increase geometrically with dosage. Breaks show preferential occurrence in the proximal region. Translocations come about by breaks followed by fusions.

There appears fairly general agreement that chromatid lesions decrease and chromosome lesions increase in number with time after radiation.* This replacement of chromatid lesions by chromosome lesions varies with the material used. Prophase takes only a few hours to develop, and even allowing for delay of development through radiation, chromosomes react as if some were unsplit, others split in interphase, and as if some had had separate half-chromatids in the preceding division cycle, assuming no delayed breakage.

After 48 hours Mather (1937*b*) observed only chromosome breaks. Sax (1938) observed no chromatid lesions between the fourth and the eighth day but found some on the ninth day. Levan (1937) reported a half-chromatid translocation which had possibly occurred in meiosis and was observed in the microspore. For three days after radiation, Marquardt (1938) observed only chromatid lesions in *Bellevellia,* so that Straub (1938*b*) concludes that most x-ray experiments indicate that the chromosome is double in structure. Geitler (1938*a*) states that x-rays merely test a peculiar kind of reactivity, and Huskins (1937) believes that chromatid breaks are decided evidence of doubleness whereas chromosome breaks are no evidence at all of singleness.

Much discussion has centered around the mode of chromosome rearrangement under radiation. This problem is probably closely related to that of normal crossing-over which is at present not understood. Two threads, each with a proximal and a distal end, break, and the four ends reunite at random into two complete threads. In crossing-over the reunion is limited to that of a proxi-

* If the radiation is applied to increasingly earlier stages of a given nuclear cycle, the effects of which are compared by observation of the first ensuing metaphase.

mal with a distal piece and to homologous threads. In x-rayed material there are no such limits. Breakage and reunion may come about either as two events, separated in time, or as simultaneous events, called "contact" method by Catcheside (1938*a*, *b*); while the proof in favor of either method is not conclusive, (Catcheside 1938*b*) most workers favor the breakage and reunion scheme which Carlson (1938*a*, *b*) believes to have seen in operation when certain akinetic fragments followed the polar movement of the kinetic elements and apparently attached themselves within the telophasic nucleus. Breakage followed by reunion is favored by Buck (1939*b*), Camara (1938), Demerec (1937*b*), Parthasarathy (1938), Sax (1938) and implicitly by Catcheside (1938). In case of the contact method it is argued by Catcheside that the high frequency of rearrangements requiring 3 or more threads in one point could occur only if the nucleus is completely filled by chromosomes. The frequency of such rearrangements would be a measure of the frequency of such contacts. If they did not fill the nucleus the high frequency of rearrangements would be evidence against the contact method. On the other hand, according to Catcheside, the high frequency of inversions in one arm relative to the frequency of interchanges between different arms demonstrates a special preference either in the fusion of breakage ends or in the grouping of original breaks through a contact or analogous mechanism.

Little is known about changes of x-ray sensitivity. According to Demerec and Kaufmann (1937), euchromatic and heterochromatic regions have the same breakage rate. Yet Sax (1938) observed that chromosomes may break more readily in their proximal regions, and it is commonly acknowledged that breaks of the kinetochore are rare. It is not known why prophase shows high sensitivity although it suggests itself that what accounts for the increased stability of interphase and division stages may be chemical cross links within the chromonemata, perhaps chemical salt linkages which allow a broken thread to heal again, whereas in prophase, once broken, the break will become effective.

The stacking up of hits in definite regions presents a strong argument in favor of some conditioning mechanism which may accompany chromosome breakage. Demerec and Kaufmann (1937) state that breaks tend to concentrate on certain chromosomes but that fusion of the broken parts occurs subsequent to the break rather than that breaks are the result of chromosome fusion as has

been sometimes assumed. Regarding genetic effects Fricke and Demerec (1937) write: "Genetic changes are not produced by direct activations within a gene but they are probably in the nature of sensitized reactions induced by the transfer of energy from neighboring activated molecules." From the discussion of Marquardt (1938) it is fairly clear that if one prefers not to assume that chromosome parts are attracted toward one another by a sensitizing mechanism one must institute a seeking mechanism whereby free ends find one another. Perhaps both are needed.

Kaufmann and Bates (1938) have shown that rearrangement may insert a reversed piece of one chromatid into the sequence of a sister chromatid—a typical example of localisation of effects. Buck (1939) has pointed out the numerical consequence of chromosome breaks and rejoins if they take place while the chromonema is in a coiled condition. If two synapsed chromosomes, or sister chromatids, coiled into a helix, are broken across two coils, the six resulting fragments may rejoin two by two in 105 different ways. Buck's work again is founded on the assumption that breaks are adjacent or propinquant. Carlson (1938*a*, *b*) believes that reunion of broken pieces may be much delayed. Superficially this conclusion disagrees with Sax's that there is no temperature effect. Yet both observers may be correct, Carlson dealing with a more rare phenomenon.

Catcheside (1938) states that "breakage" and "contact" hypotheses both fill the requirements if it be supposed that structural rearrangements chiefly follow breaks occurring in *nearby positions*. The pioneer work of Husted (1937) postulated that breaks and reunions in *adjacent parts* of helicated threads give rise to knots, loops, rings and inversion such as were found by him.

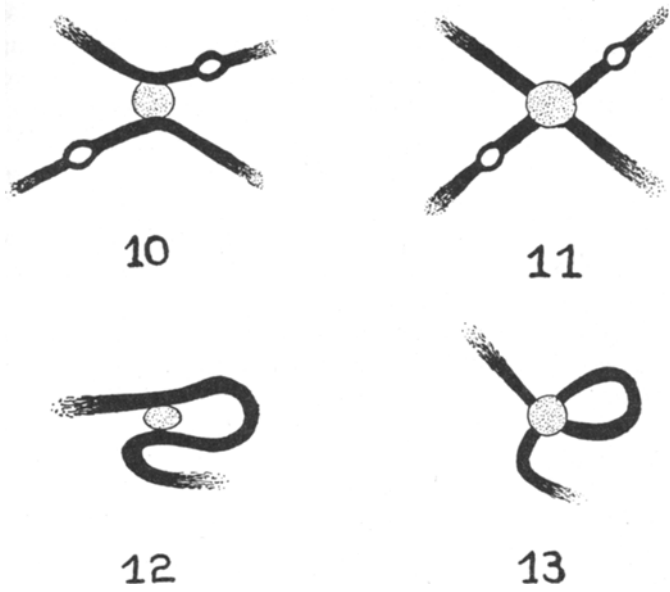
According to Stadler (1938), Stadler and Uber (1938) and Muller and Mackenzie (1939), ultra-violet rays cause fewer translocations and gross rearrangements than x-rays. Also evidence obtained by Jones (publ. in prep.), based on endosperm mosaics, shows that with increasing doses of x-rays, translocations and rearrangements increase faster than the corresponding counts of fragmentations without reunions would suggest (See also Sax, 1938, *506*). In view of this, the regional accumulation phenomenon, and in view of the fact that lesions increase with the 3/2 power of dosage it appears suggested that a second order process is at least facultatively involved.

A tentative diagram satisfying the various requirements may be devised. Under the influence of radiation chromatids and chromosomes are broken by single hits. In addition, "affected areas" may be formed independently at random and may thus be located upon or in propinquity to a chromosome. If one such area is formed on a given chromosome, this increases the likelihood that more such areas will occur on the same chromosome. Proximal parts of a chromosome may form such areas more easily than distal parts. Extra chromosomal "affected areas" will attract neighboring chromosomes. Intra chromosomal areas will attract adjacent parts of the same chromosome (diagram 12). Further radiation may break all threads abutting onto a sensitized or affected area by means of single or multiple hits (Sax, 1938). Now we have multiple ends held in mutual proximity by an affected area. The ends may group themselves symmetrically around the central sphere of disturbance (diagrams 11 and 13). If the central area diminishes in size, free ends will be pulled together. They will unite at random in pairs. Why and how this is accomplished we do not know, but it is as if the free ends of chromonemata sent out search threads which when meeting other search threads contracted and tied their respective axial gene-threads together (diagrams 14, *a, b, c,* p. 594).

Such a picture as the foregoing may hold for prophase and interphase, but during metaphase and prometaphase chromosomes are so well insulated by their matrices that translocations between them do not take place easily. If, however, meiotic chromosomes are stretched across the plate by the existence of more than one kinetochore and broken by stress, then they may react as if the two threads within each broken end united (McClintock, 1938*a*). Thus the findings of Sax and McClintock supplement each other towards the general statement that where a chromosome is broken shortly before or after metaphase it may react as if the matrix were capping over and healing the wound in such a way as to make the two free sister ends continuous (diagrams 15, *a, b, c*). Where a matrix is not present joins of this type are unlikely. From this outlook the end genes of Belling or end bodies (*cf.* Raffel, 1939) take on the mechanical significance of maintaining a necessary amount of repulsion between sister threads so as to counteract the capping effect of the matrix and the tendency to join which apparently exists in the new ends of freshly broken threads. Paren-

thetically, the repellent property of the end bodies is often expressed in the frayed appearance of chromosome ends.

With regard to the rejoining method, Camara (1938) has pointed out that if coiled sister chromatids undergo simultaneous translocation by the cross break and rejoin method, it would result in sister threads not being broken at the same genetical level. Also the joined end fragments, where such are produced, should have different chromatid lengths. The facts argue against a mechanism as simple as that underlying Camara's presentation.



Figs. 10 to 13: Tentative diagrams of the effect of "affected areas" in chromosome breakage under x-radiation. The dotted body in the center of each figure represents the "affected area." In Fig. 10 parts of different chromosomes are united by an "affected area" in Fig. 12 different parts of the same chromosome are united.

In Figs. 11 and 13 multiple breaks have taken place in the chromosome pieces abutting on an "affected area." It is suggested that the free ends will be pulled together at random by surface forces of the "affected area" which will diminish in size with general "recovery." (See text p. 576.)

GENERAL CONSIDERATIONS CONCERNING GENES

The problems arising from radiation are directly dependent on the molecular structure of chromosomes, concerning which several suggestions have been made. Gulick (1938) believes that the

chain of genes may be carried in the chromonema and held in alignment by chemical bonds arranged in a nonpolar pattern. Either the gene molecules are basic proteins held in place by longitudinal nucleic acid molecules, or they are nucleoproteins some of whose nucleic acids straddle from gene to gene, or they may be nucleoproteins alternating with a basic protein-filling substance to which they are bound on both sides by their nucleic acid valencies. Thus the chromonema is not to be explained by the hypothesis of longitudinal polypeptides possessing amino acid and carboxyl groups at opposite ends.

Schulmann (1938) emphasizes the changes in distribution of nucleic acid during the mitotic cycle, its rise before division, and its fall after. The more active the metabolism the more active are the changes in the nucleic acid content. The latter principle was first pointed to probably by Caspersson and Schultz (1938) who suggested that the property of a protein which allows it to reproduce itself may be its ability to produce nucleic acid. Frey-Wyssling (1938) believes with Wrinch that the polypeptide chains run parallel with the thread axes but that the first suggestion of Wrinch put forth in 1936, namely, that the nucleic acid molecules stand at right angles to the thread axes, must be changed to a picture in which they run parallel with the main axis. Signer, Caspersson and Hammersten (1938) have shown that the nucleic acid molecules are 300 times as long as wide and show negative double refraction, and this has been attributed to the plane of the purine rings which are at right angles to the axis of the chromonema. Gulick's suggestion that between the genes proper there is a nongenic basic protein-filling substance has many advantages from the speculative angle. All mechanical features which are so outstandingly uniform throughout a chromosome set, as well as general reactions such as sensitivity to radiation, the reunion reaction, temperature responses and general coordination, could be attributed to the presence of this non-genic filling substance.

Gottschewski (1937) would actually like to think of the genes as being detachable from the chromonema in certain cases. Thus he found that in over 20 generations a deficiency of chromomeres for a certain notch gene could not be detected. Genes appear also to be far from autonomous with regard to their propensity to mutate. Thus Demerec (1937*a*) found the mu-F gene to affect the gene environment during the development of germ cells so as to

bring to a threshold a condition at which changes in various genes occur. This corresponds in principle to the minute genes governing somatic crossing over (Stern, 1936) and with the Dt gene of Rhoades (1938*b*) which labilizes the color gene a_1 so that it may change to A_1 about a thousand times more frequently than normal. Here asynaptic genes of corn and sticky genes rendering the chromosomes sticky, as well as the recently described gene for an apolar first meiotic spindle in maize (Clark, 1939), must be mentioned. In all cases a single gene appears to govern the reaction pattern of the entire nucleus or at least large parts of it. Is it not easier to imagine that a gene is producing such a governing principle "*g*" and that "*g*" will find identical "mechanical units" at regular intervals on the gene string with which to interact, rather than to imagine that each and every gene besides its specific genetical purpose must have a mechanical function strictly identical in all mechanically corresponding parts of all chromosomes?

With regard to position effect one may say that this phenomenon by no means invalidates the corpuscular integrity of the gene. The expression of a specific gene corpuscule in terms of visible characters is modified, however, by the position of the corpuscule in the chain of genes composing the gene thread. Jones (1939*a*) found among the irregularities occurring in maize endosperm, due to chromosomal irregularities, raised and depressed areas not necessarily linked with color changes. These are attributed to nonlethal chromosomal unbalance or to a physiological change at specific points of chromosome breakage and reattachment.

Jones (1939*b*) relates a marked case of position effect when he writes that in corn endosperm tissue, where spontaneous translocations are observed, the relocated C region (C being a dominant color gene) not only fails to function as usual but prevents normal action of the other C allele. In this way it acts like a partial inhibitor of color.

Gustafsson (1938*a*) has gone far towards an approach upon the physiology of the gene and believes that a hypothetical sequence of genotypical size may be established in chlorophyll mutants in such a way that the genes represented by larger corpuscules are supposed to produce an allelic series more readily than smaller genes. Genes may also differ in attraction toward their neighbors. He further holds that micromutations arise more readily in dry than in damp nuclei, whereas "alboviridis," assumed to be of large size, arises in

nuclei with a high water content which in consequence give more chromosome aberrations and are more easily killed. Gustaffson (1938*b*, *c*) also believes that the water balance of nucleic acids is chiefly responsible for the difference between mitosis and meiosis. Undoubtedly, hydration is one of the most obvious features of cellular and nuclear changes, but it is hardly a self-governing principle of autonomous nature. In radiation of plant material it is scarcely possible to differentiate between a "wet" and a "dry" gene without involving cellular function and thus differential sensitivity due to mitotic stage.

Many comparisons have been drawn between genes and virus molecules. Lindegren (1938) suggests that the chromatin envelope of the gene protects the gene from the cytoplasm, as the former, if loose, might devour the latter or the latter in turn might inactivate the former. Gowen (1939), studying tobacco mosaic virus, found that medium wave lengths of radiation were most effective and that the presence of water had no effect on the process of inactivation. The former and perhaps both findings distinguish at least superficially genes from the virus molecule.

Inactivation by ultra-violet light (Price and Gowen, 1937) follows an exponential curve—as chromosome breaks—and here also wet and dried juice reacted identically. One may perhaps conclude the chapter on the gene with a quotation from Gates (1938): "All that we really know about the so-called genes, is that they represent a difference, arising in the germ plasm through a mutation,—as I first pointed out in 1915."

CHROMOSOME STRUCTURE AND EVOLUTION

The bearing of chromosome structure on evolution has been the subject of a large series of papers and two recent books (Dobzhansky, 1937; Darlington, 1939), so that this subject requires no further reviewing. Of very recent date one might list the work of Stebbins (1938) on cytological characteristics of different growth habits in dicots in which it is brought out that the basic numbers are higher in the woody group mostly carrying small chromosomes, many being of polyploid origin. A relatively high stability and relatively high chromosome compatibility is correlated with their relative stability in habit. Babcock (1938), Babcock, Stebbins and Jenkins (1937) and Babcock and Cave (1938) have exhaustively treated the evolution in the Crepidinae.

Woods and Bamford (1937) have described chromosomal evolution concomitant with the phylogenetic development in tulips. Blakeslee, Bergner and Avery (1937) have dealt with geographical distribution of chromosomal prime types in *Datura*. Dobzhansky and Sturtevant (1938) have presented an ingenious method of analyzing the historical sequence of chromosome rearrangements which may be applied to strains as well as to species. The method consists of comparing inversions which may be single, and if multiple, may be "independent," "overlapping" or "included." Sturtevant and Mather (1938) have shown how, if a chromosome exists in a population in two sequences, two distinct lines of descent will be established. The two lines will acquire different deleterious recessives. Thus the sequence heterozygote will be at a selective advantage. But heterosis and recombination are in opposition. Once a sequence change has taken place in one chromosome, further changes in that chromosome may increase recombination. The chance of inversions in other chromosomes is reduced by the fact that they will counteract recombination. After reciprocal translocation the heterozygote has reduced fertility due to irregular segregation. Inversions within inversions may have the same effect (Sturtevant, 1937; 1938*a*, *b*). Rahn (1939) points out that evolution depends on chemical changes within the genes. Evolution will thus be speeded by temperature increases, especially in forms with a rapid reproduction and a small body size. There are eight times as many species of reptiles in tropical climates as in moderate ones, against a ratio of 2:1 for mammals. In bacteria variation is so common that in several group species definitions are arbitrary.

SYNAPSIS

Perhaps the most spectacular progress in cytogenetics has been made recently through the intelligent analysis of synapsis and crossing-over. From this has come a more thorough understanding of the evolutionary role of chromosome rearrangement. Much as this work means in terms of a genus or even a family investigated, the principle, as far as chromosome structure viewed from the angle of cell mechanics is concerned, is always the same, even if it may reach very complex refinements presenting intricate puzzles to the investigators, as is true of *Oenothera, Campanula, Datura, Pisum* and certain polyploids. Recent papers in this field

should be quoted for reference: Darlington (1939*b*), Dobzhansky (1937) (text books), Bennett (1938), Bridges (1937), Darlington (1937*b*), Darlington and Gairdner (1937), Emsweller and Jones (1938), Haga (1937), Knapp (1937), Levan (1939*a, c*), Matsuura (1937; 1938*a, b, c*). Ribbands (1937), Sansome (1938*a, b*), Sax (1937*a, b, c*), Sturtevant and Mather (1938), Stebbins (1938*a*), Sutton (1937), Thompson and Thompson (1937), Upcott (1937), Woods (1937).

These papers cover the general field and special discussions of *Sphaerocarpus, Lilium, Tulipa, Tradescantia, Trillium, Paris, Allium, Oenothera, Pisum, Paeonia, Gossypium and Drosophila.* The encyclopaedic treatments by Darlington and Dobzhansky make further discussion of this subject unnecessary. A comprehensive study has been made of environmental effects on pairing by the Black Forest School of Oehlkers. These studies have been competently summarized by Oehlkers (1937*a, b*). Some workers of this group are Ernst (1938), Haselwarter (1937), Kisch (1937), Marquardt (1937*a, b*) and Straub (1937; 1938*a, b*). Through changes in temperature, humidity and nutrition, synapsis and the formation and terminalisation of chiasmata may be variously affected. Generally speaking, pairing and chiasma formation and terminalisation may be adversely affected by conditions departing from the normal. It is important that the changed conditions affect the cells at the critical stage. In a more general way Gustafsson (1938*b, c*) has approached the problem from a different angle. Meiotic development in *Hieracium* can be counteracted and transformed into mitotic development by substances which in pollen mother cells of *Hieracium* are released from the degenerating tapetal cells. Huskins and Wilson (1938) produced three abnormal types of pairing in *Trillium* by means of temperature. They may be distinguished as asynaptic with chromatids close, asynaptic with chromatids not close and desynaptic with early terminalisation.

Stein (1936) reported somatic reduction in chromosome number following somatic synapsis in the floral region and especially in the male archespore of a mutant *Antirrhinum* induced by radium radiation. The original cells are polyploid, and in early prophase the chromosomes approach each other. Before prometaphase there is tight parasynapsis; in metaphase the chromosomes are highly con-

tracted. One might say that here meiotic conditions have been projected into a stage preceding meiosis. Levan (1939*a*) observed what appeared as somatic crossing-over between "sister" chromatids of diplochromosomes induced by growth substance. Stern (1939) has restated that somatic crossing-over is exclusively between homologous loci. Unbalanced exchanges may survive, but in material not carrying inversions they were not found to arise. To understand the conditions of meiosis and synapsis is not only of fundamental theoretical importance but also a practical problem which has great consequences in plant breeding. Dealing with artificially doubled amphidiploid tissue and having intercrossed such amphidiploid forms, a method which would induce reduction would give one a somatic series of halved amphidiploid segregants which can not be obtained by any other means.

Important research is in progress concerning the time and place of crossing over with regard to the chromosome. Eventually, this work will help to understand certain peculiarities of chromosome structure, but any conclusions at this time may appear premature. Lindegren and Lindegren (1937; 1939) have traced the non-random crossing-over in *Neurospora* in which individual chromatids can be followed directly in the haploid phase of the fungus. They find that the ratio of 2:3:4 strand double exchanges is as 55:29:16 instead of as 25:50:25. Thus exchanges between tetrads are usually multiple, symmetrical about the kinetochore and preferentially two-strand exchanges. A special theory involving concepts of Belling and Darlington is suggested whereby unsplit leptotene threads synapse at the kinetochore and form relational coils. New chromeres then form beside the old ones and cross-overs occur at points of overlap between two new sister strands. Three- and four-strand exchanges come about by occasional sister strand exchanges between loops. Although the Lindegrens (1939) have recently shown that this scheme holds for the second chromosome as well as for the first, it can not be given general consideration because in other organisms crossing-over is at random and there is absence of positive or negative chromatid interference. Recent data from McGill on chiasmata in *Trillium* indicate that twisting is not directly related to crossing-over since in a paper by Huskins and Newcombe (1939) the existence of negative chromatid interference is demonstrated. Weinstein (1939) has shown why in *Drosophila* crossing-over between sister chromatids is excluded and

why recurrence must be random (recurrence refers to the number and nature of exchanges at each level and their relation to exchanges at other levels). Mather (1937*a*; 1938) suggested that the first or proximal chiasma forms at a mean distance from the kinetochore which is correlated with the length of the chromosome arm. The second chiasma forms at a mean distance from the first which is probably constant throughout the chromosome complement. The latter or interference distance is not the same as the former or differential distance. Crossing-over tends to occur in definite positions as a result of a special time and a special sequence in formation of chiasmata. Sansome (1938) points out that the special sequence of forming chiasmata is not established genetically. Charles (1938) has published extensive evidence not in agreement with Mather. According to Charles (1938), the average positions of the exchange in tetrads where only one exchange occurs is approximately the midpoint of the chromosome. Where two exchanges occur these are approximately at the midpoints of left and right halves of the chromosome. For any particular position of one exchange in two-exchange tetrads the average distance to the next exchange is about $\frac{5}{8}$ beyond the first exchange. In three-exchange tetrads the average positions are approximately the midpoints of the left, central and right thirds of the chromosome. The data do not support Mather on the temporal seriation of chiasmata, starting at the kinetochore.

While in all organisms the basic event of crossing-over, that is, the breaking and rejoining of chromonemata, probably occurs in the same fashion, typical of gene-strings in general, it is quite possible that this event is touched off by a variety of different trigger mechanisms. Relational coiling may be the instigating cause in some forms; in others there may not be a grossly mechanical principle to condition crossing-over. One might speculate on a certain type of random unbalance from non-simultaneous internal origin of sister half-chromatids during zygotene, setting up at these points an unsaturated state akin in type to the pairing principle of precocity speculated upon by Darlington. It is rather remarkable that for synapsis as such so far no tempting speculation has been suggested. Snell (1938) mentions the attraction of like electric currents, crystal formation and the miscability of identical liquids. Agglutination was suggested by Bridges, and Van der Waals' forces are given a place by Waddington (1939).

None of these general forces will do, however. It is as if genes —which normally attract enough feeding or building material probably in preformed blocks from their surroundings, the matrix of interphase—were during leptotene suddenly starved for such building stones of a peculiar kind and thus started to prey on one another. In this picture it is assumed that even during interphase chromosomes are surrounded by some sort of karyomeric surface. The breakdown of this during leptotene, or interphase in *Diptera,* would initiate pairing.

It has been shown that under certain "normal" conditions the individuality of chromosomes may be seriously upset (Jones 1938*a*, *c*). In corn endosperm interchange between non-homologous chromosomes is indicated. Reciprocal interchange is shown by paired spots, one of which exhibits the loss of one dominant gene, the other and adjoining spot the loss of another gene, the two genes being located on different chromosomes but removed simultaneously. In a few cases the gene removed from one cell was added to another. Different results were obtained depending on where the break occurred and where the fragments were relocated. In this tissue a condition must naturally be reached which closely resembles a gene environment artificially created by x-rays. Perhaps the physiological basis of these conditions may soon be elucidated.

Cooper (1938) suggested that in meiotic pairing the units have a bilateral organization. In polytene chromosomes the pairing units would be radially symmetrical; such mental imagery is helpful for purposes of logic. One might add to this picture of the longitudinal pairing surface that of the transverse non-specific joining surface which must have quantitative features of being attractive without narrow qualitative limits.

Doubt has been cast recently on any too hasty assumption of secondary pairing by Catcheside (1937*a*), Heilborn (1937) and Propach (1937). Catcheside investigated *Brassica* and believes the basic number to be six. However, secondary pairing is dependent on the diakinesis positions of bivalents relative to one another. Large size and high numbers hamper secondary pairing. Structural dissimilarity weakens the chance of secondary pairing. Apples show it well, pears do not. According to Heilborn, secondary pairing is a misnomer and should be called secondary association only. It consists of a mechanical sorting out according to size and mass through forces of nuclear division. It supplies infor-

mation on the smallest possible number of distinct classes of homologues. The paper of Propach is even more critical than the foregoing. Propach tried to use secondary pairing as a criterion of distant homologies in *Solanum*. Not having been successful he plotted data of Müntzing on *Solanum* and of apples after Heilborn, using valency as one axis and chromosome number as the other. Thereby valency and frequency of secondary pairing groups appear as a function of chromosome number. Thus the condition of quadruplicate tetrasomy, triplicate hexasomy, in the apple, and probably several other data of the literature would deserve a statistical treatment according to Propach's procedure before attaining classical significance.

METHODS AND PHYSIOLOGY

Although a great deal of the success of work on the structure of chromosomes depends on methods, very little systematic experimentation has been done in this direction, without doubt in part because genetically minded cytologists do not wish to think in terms of physiology and physiologists prefer not to interpret genetical units. The recent text of Zeiger (1938) may help to fill this gap.

On staining, especially on the use of the Feulgen reaction, a twenty page bibliography by Milovidov (1938) has just appeared. Whitaker (1939) emphasizes the use of Benda's fixative preceding the Feulgen technique. Yuasa (1937) described the relation of chromic acid to the outcome of Feulgen's stain. A differential method of showing chromonemata involving hydrolysis was given by Darlington and La Cour (1938).

The Feulgen method combined with light-green after a treatment in 5 per cent sodium carbonate counterstains nucleoli green (Semmens and Bhaduri, 1939). Drawert (1937) found that nucleoli have a relatively high pH value of 5 with chromosomes around 3 and the spindle between 4.5 and 5.

With as much obvious emphasis on a qualitative determination of nuclear constituents on a microscopic scale, the extensive work of Caspersson (1936; 1937) appears to offer even more direct methods of determining cell constituents. It is to be hoped that differential absorption methods of determining nucleic acid constituents can be developed into a general technique.

Several modifications of permanent smear methods have been suggested; some of them are summarized by La Cour (1937).

Bridges (1937) replaces the acetic acid used for staining, very gradually by supplying alcohol vapor in a closed jar. Hillary (1938) used dioxane after removing the stain with 50 per cent acetic acid, and recommends corn syrup and acetic acid for separating and teasing. For the latter purpose Heilborn (1939) has constructed a special roller and he also introduces a slight glycerine coating on the under side of the cover glass which reduces sticking where the cover glass must be removed to make the slide permanent. Jeffrey (1937*a, b*) describes a method in which the tissue is fixed in very thin slices assembled on cards and sectioned from nitrocellulose. Permanent methods for mounting of pollen tubes are described by Beatty (1937), Eigsti (1937*b*) and Newcomer (1938). The combined staining, fixing and mounting medium of Zirkle (1939) deserves serious attention.

Most changes in chromosome structure can be described in terms of relative hydration, and a series of Japanese papers uses this approach throughout. According to Wada (1937), the ground substance of the chromosomes swells under ammonia; under heavy doses chromatin threads swell and finally destructive peptization takes place. Under fumes of butyl alcohol chromosomes may temporarily become narrower. A slight shrinkage also takes place under chloroform and ether fumes in doses not exceeding reversible effects. Wada (1938*a, b*) and Kuwada (1937) have followed the hydration and dehydration caused by solutions of different tonicity. Unravelling of coils may be brought about by hypotonic solutions and may be partly reversed by hypertonic solutions. Shigenaga (1937) produces amphidiploid nuclei by stopping metaphases hypertonically or with drugs and then applying hypotonic solutions or water. Yamaha (1938) determined the rH of chromosomes as 15.8 at a pH of 7.0 and also tested several vital dyes. Matsuura (1938*c*) uses tapwater as a pretreatment to obtain chromonematic coils in *Trillium*. Kuwada, Shinke and Oura (1938) summarized the various solutions which may be used to produce the artificial uncoiling of the chromonema.

Dawson (1937) has shown that during secretion the structure, volume, form and reaction of nuclei are so much changed that nuclear participation in the act of secretion is strongly suggested.

Churney and Klein (1937), Yamaha (1937) and Kamiya (1937) have studied kataphoresis. Chromatic elements migrate to the anode and this migration is increased if acid vital dyes are intro-

duced. - Churney and Klein believe the nucleus as a whole to be positively charged. Kamiya considers the cytoplasm differentially polarized, the division figure being passively transported. It is not ascertained whether electric currents such as were used in the kataphoresis experiments harmed the chromosomes.

Changes of age and death under varying circumstances have been investigated. Thus ageing of pollen will result in bridge chromosomes observed by Barber (1938) in *Paeonia* and *Kniphofia*. They are suggested to have arisen from fusion of sister chromatid ends during the prolonged interphase. Cohen (1937) has shown, not for the first time but with great thoroughness, how the reticulation in fixed nuclei originates through collapse and fusion of chromonemata. Reversible homogeneous and chromonematic structure was achieved by basic and acidic treatment, and the critical interpretation of optical images is discussed.

Ries (1937) establishes a continuity of chromatic structures on the grounds of plasma physiology. Reversible peptization and dehydration of acid nuclear proteins is the basis of vital staining. Acid nuclear proteins are protected by a solvation coating. A nucleus may appear to show a basic reaction and may not stain with basic dyes although acid proteins are present. Peptization which initiates death changes can be staved off, and the protein lipoid complexes may be stabilized by raising the alkalinity of the cell. Pischinger (1937) points out that nucleic acid will under acid fixation become electrically discharged and dehydrated. Under shrinkage it will be precipitated in a reticulate fashion whereby free existing surfaces are preferred. Histones will be dissolved. Other mixtures which will not separate nucleohistones will give rise to charge changes, dehydration, coacervation and peptization. Conklin (1938), Haselwarter (1937), Matsuura (1937*b*), Sax (1938) and Straub (1937) investigated the influence of temperature. It is not known exactly which reactions in the nucleus are the primary ones affected by temperature, so that so far it is possible to record only the large scale processes in which mutual timing and orientation appear to suffer most. Conklin studied *Crepidula* under low temperatures. The hyaloplasm clots first; next, vortical currents are suppressed; then, spindle fibers, asters and chromosomal division are hindered. Lastly, telophasic nuclei fail to become interphasic. Resting nuclei and nucleoli may continue to grow and become enormous. Sax, using temperature jolts, obtained in

Tradescantia all conceivable forms of mitotic and chromatic irregularity so that he concludes that the effects are in many respects like those produced by narcotics, osmotic pressure, mechanical injury, regeneration, insect and fungus invasion, x-rays, ultra-violet rays, genetic factors and ageing. This may be true on a morphological and general basis, primarily because dividing cells have a limited number of morphologically accessible processes, all of which can be eventually affected by almost any type of the agents listed. Emphasis on specificity of distinct agents and distinct quantities of such agents must follow a general survey.

Matsuura (1937*b*) especially emphasized the desynaptic effect of high temperature. The work of Haselwarter and Straub was mentioned earlier. Kostoff (1938*b*) and Beams and King (1938; 1939) studied the effect of centrifugal force. Kostoff obtained translocations as well as amphidiploidy after treatment of seedlings. Beams and King give an exhaustive summary of this subject.

The study of the effect of drugs on the behavior and structure of chromosomes has not received the attention this field deserves. Nuclear or even cellular toxicology requires future attention. Levan (1938; 1939*b*) has made probably the most comprehensive study of the effect of colchicine on plant chromosomes and their structure. References to this subject can be found elsewhere (Ruttle and Nebel, 1939). In addition, Levan (1939*c*) found that plants treated with growth substance—indolbutyric acid —doubled their chromosomes by multiplication during the resting stage. This observation is of extreme theoretical import, as it shows for the first time that a process first observed by White in x-rayed grasshoppers and then inferred by Geitler to exist in *Gerris* may also occur or be induced in plants. Levan practically eliminated the possibility that the diplochromosomes which he observed might have formed by failure of the spindle—as under colchicine— and of successive pairing of sister units. The doubled cells of Levan were, however, hypertrophied and mature cortical cells. Perhaps this type of doubling should be likened to a process of regulation in which the nuclear volume follows the volume of the cell, whereas in colchicine the nuclear volume leads the cellular volume.

Sass (1938) reported a severe influence of ethyl mercury phosphate on mitosis, giving rise to multiplar spindles, irregular and incomplete separation of split chromosomes at anaphase, failure of plate formation, and the occurrence of restitution nuclei. Bind-

loss (1938) suggested that in certain hybrids showing heterosis nuclei might be enlarged.

SPINDLE AND MITOTIC APPARATUS

In recent papers on the spindle and extra-chromosomal agents, as these affect the structure of chromosomes, genically controlled effects have received considerable attention. Clark (1939) has described a simple recessive gene from corn pollen treated with ultra-violet light which caused the first meiotic anaphase spindle to be "divergent," leading to scattering of chromosomes and 30 to 60 per cent of pollen abortion. At the end of the first meiotic division a number of temporarily viable micronuclei are formed. The one containing or consisting of chromosome 6 may have an organized nucleolus. Microspores with at least a complete set of chromosomes, although these may be in separate nuclei, are capable of apparently normal growth and can undergo the first microspore division. A single tube nucleus and a single generative nucleus may result from a multinucleate spore through a directed orientation of the several spindles produced by separate nuclei. This brings to mind the work of Schrader (1932) according to which in several coccids spindles normally are divergent, but division is nevertheless normal. The material of Clark is in distinct contrast to the more common breakdown of micronuclei, as in asynaptic condition, trisomic segregation and the fragments resulting from inversion cross-overs (*cf.* Emsweller and Jones, 1938). Darlington and Thomas (1937) have described a related abnormality encountered in a trisomic *Lolium* cross. In this case, however, phragmoplasts are occasioned to function between micronuclei forming walls. The second division may be irregular within each cell, and the spindle may be regular, divergent or "unattached" which means that it is beside and in no immediate connection with the chromosomes. In the absence of centrioles or centrosomes this is a remarkable case. An opposite extreme is reported by Smith (1938) in a triploid *Impatiens* where in spite of the triploid condition of the genome behavior in meiosis is exceptionally regular.

Special reference must be made to the work of Jones (1937; 1938*a, b, c;* 1939*a, b*) (see also above) who is investigating genetically and cytologically the embryo and endosperm characters in corn. It appears that the condition of triploidy (in the endosperm) and the physiological conditions of this tissue render this tissue

subject to non-aequational mitoses and to spontaneous chromosome rearrangements. The tissue being not subject to routine cytological technique, detailed information on the nuclear phenomena involved must be referred to the future. All changes may occur spontaneously but may be increased in number by x-radiation.

Jones (1937) writes that by unequal mitosis atypical growth is brought about by the removal from or concentration in certain cells of growth regulating substances which are chromosomally controlled. This unequal mitosis may be induced by external agencies or by inherited defects of the mitosis mechanism transmitted in part by the female parent. Swezy (1937) showed spontaneous alteration of chromosomes in *Crepis*. The gains in a complement could not always be matched with corresponding losses in other parts of the plant. It seems understandable that cells with certain losses were less viable than those with corresponding gains. Beadle (1937) introduced the gene for sticky chromosomes into various plants and found that these resembled somewhat x-rayed plants. Somatic elimination of chromosomes and chromosome parts was frequent so that rearrangements were observed with an incidence of 1 in 64 as against 1 in 594 in normal plants. The mutation frequency appeared as 1 in 49 as against 1 in 379 in the normal. It thus appears that "stickiness" in this case is synonymous with a certain proneness to break and recombine which corresponds with what one observes after radiation. Is then the abnormal quality of the chromosome surface which normally repels, or at least sharply separates chromatids not in the same chromosome, not perhaps in all cases of "crossing-over" the necessary premise or perhaps even an expression of the only premise required leading to internal re-alignment, the outcome of which may be repair or rearrangement? Fabergé (1937) reported a genetic factor for male sterility in *Lathyrus* which causes cell division to get out of step with the nuclear cycle so that chromosomes in metaphasic condition are seen within a nuclear wall. Terminalisation and separation of bivalents is also typically affected. Upcott (1937*b*) finds that in pollen-sterile *Lathyrus,* development of the chromosomes is delayed relative to that of the spindle. There is also greater spiralisation and greater terminalisation. In a series of papers from Cold Spring Harbor (Satina and Blakeslee, 1937*a, b;* Satina, Blakeslee and Avery, 1938), the workers described chromosome behavior in triploids of *Datura* and plotted the preferential survival rates of

distinct gametic types, in the male and female gametophyte and in the resulting zygotes. No special mechanism for "regulation" outside of preferential survival was found. Compared with random expectation there was a 100-fold excess of 1 *n* microsporocytes observed in the first microsporic division, a 280-fold excess of 1 *n* female gametophytes, and a 3000-fold excess when the zygote or the next generation was considered. Thus the natural "inaccuracy" of the spindle mechanism "allows" of greater fertility than a normally more "accurate" spindle would.

Eigsti (1937*a*), in a comparative study of male gametophytes in *Tradescantia reflexa* and *Lilium canadense,* found that generative nuclei may produce gametes by amitosis or partial amitosis. Male nuclei may form from prophase without organizing an equatorial plate. Concerning the nature of the spindle Northen (1937) reported that, according to results from centrifuging, only the heavier components of the nuclear sap may go to form the spindle; lighter components are liberated into the cytoplasm. Beams and King (1939) have shown how after centrifuging, a cell-plate can develop in enucleated cells. Obviously, then, the spindle can not be claimed to be a product of the chromosomes and not even partly *subject to their presence.*

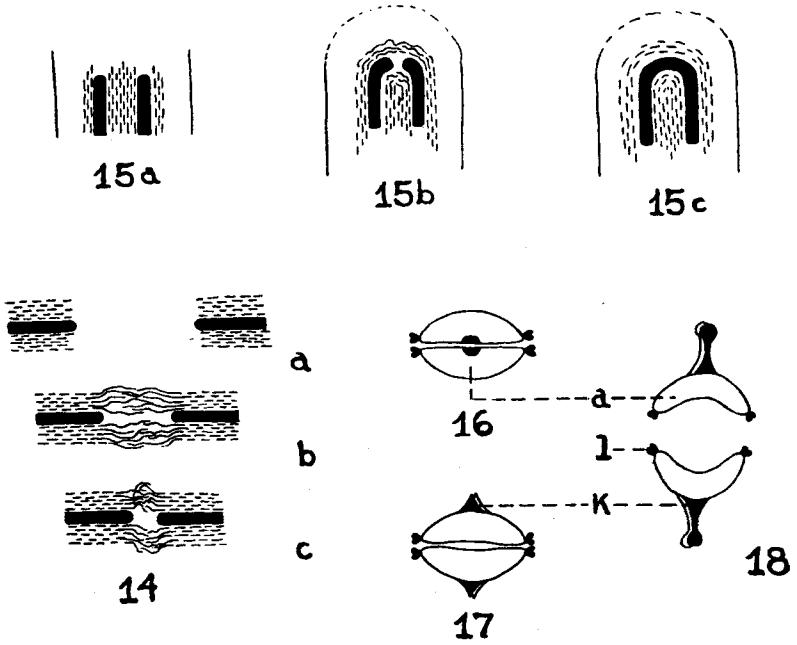
Recent work by Schneider (1938) has shown vortical currents to be present in plants during mitosis, sweeping from the poles to the phragmoplast along the outer edge of the division figure and thus similar to such currents recently described by Chambers (1938) in sea urchins. Similar movements have been described in the now classical work of Bütschli, Erlanger and Spek (see Wilson 1928, *194–198*). Wassermann (1938) considers the reality of spindle fibers as established and considers that a progressive pectization progressing from the poles establishes chains of micellae of higher viscosity interspaced with a phase of lower viscosity. While it is mostly the kinetochore which interacts with this process, occasionally other parts of the chromosome may take part, as shown by Carothers (1936) and Schrader (1936). On seeing the large centrioles of certain polymastigote protozoa (Cleveland, 1938), one might ask whether these large structures might not yield supplementary evidence on the general subject of chromosome structure. They may have, in common with chromosomes, the filiform shape, certain staining qualities and the ability to divide by fission. On the other hand, their mode of origin, their occasional branching and their presumable voidness of genetic factors set them apart.

THE KINETOCHORE

Each chromosome, to be functional, apparently requires a kinetochore (diagrams 16 to 18). This is a compound organ of the chromosome located interstitially or occasionally terminal. It consists essentially of three parts: a central achromatic body (*a*); the chromatic kinetic bodies, Leitkörperchen, chromatic spherules or minute granules (*k*); and, I believe, the often chromatic connecting chromomeres (*l*) of the chromonemata with the central achromatic body. In addition, it may have a pellicle, matrix, coat or simply a surface which in its reactions is distinct from those of the chromosome proper. This description is, however, tentative. It was found by Schrader (1936) that the central achromatic constituent of the kinetochore takes chondriosome rather than chromatin stain in the Kull and Benda methods. Upon further differentiation Schrader found a "commissural cup" carrying the chromatic kinetic body or bodies. Thus the central achromatic body may exist as a protrusion under certain conditions and in certain forms or as a cup in others. It is suggested that the image of protrusion is linked with a chromonematic condition of the chromosome, whereas a cup is shown in the presence of matrix. Schrader also suggested a possible connection between the centriole and the kinetochore due to similar staining affinity.

If the kinetochore is broken by x-rays (McClintock, 1933) or breaks naturally (Darlington, 1939), I assume that the kinetic body or bodies will be regenerated in that part of the kinetochore not having retained any after breakage. With regard to the lateral joining chromomeres which I suggest facultatively as a part of the kinetochore, it is assumed that these also may be regenerated after loss because they appear indispensable in established forms with "terminal" insertion regions, and yet, as Darlington (1939*a*) has shown, they may not be present when such a chromosome with a terminal kinetochore first arises. Diagram 16 shows this body in prometaphase in polar view; diagram 17 in side view; diagram 18 is a side view of early anaphase. It is suggested that all 3 parts of the kinetochore are potentially multiple but may also appear single, depending on methods and materials used. The multiplicity of the kinetic bodies was shown by Nebel (1935), and of the kinetochores by Schrader (1936) and by Darlington (1939*a*). While it has thus been maintained that each chromonema has essentially its own

kinetochore (Nebel, 1937*c*), the majority of workers have adopted the concept that as long as several chromonemata are held together by one kinetochore the kinetochore is unsplit or single. Certainly, however, the kinetic bodies are multiple before metaphase, and it is suggested that there is one for each existing chromonema. Mechanically the kinetochores appear to vary independently of their respective chromatids or chromosomes. Towards x-rays they are more resistant (*cf.* McClintock, 1933). They may, however, show autonomous propensity to break independently of the chro-



Figs. 14 and 15: Tentative diagrams of how broken chromatic elements may be reunited. Fig. 14*a*: Broken chromatid ends. Fig. 14*b*: Matrical material proliferates beyond ends of broken gene threads. Fig. 14*c*: After meeting, proliferated matrical elements in broken area contract thus uniting broken ends of the gene string. Figs. 15*a* to *c* illustrate the same phenomenon in broken ends of sister chromatids. Fig. 15*a*: Matrical material proliferating. Fig. 15*b*: Matrical elements uniting. Fig. 15*c*: Ends of sister gene threads united. (See text p. 576.)

Figs. 16 to 18: The kinetochore of a somatic metaphase chromosome in polar view, Fig. 16, in side view, Fig. 17. Fig. 18: The same kinetochore in anaphase. The kinetochore is believed to be at least four-partite at metaphase carrying four minute granules (*k*) which are pulled out into bodies shaped like nine-pins at late metaphase (Fig. 18). The main part is called the central achromatic body (*a*) each sector of which may carry two chromatic connecting chromomeres (*l*) one at each end. (See text p. 593.)

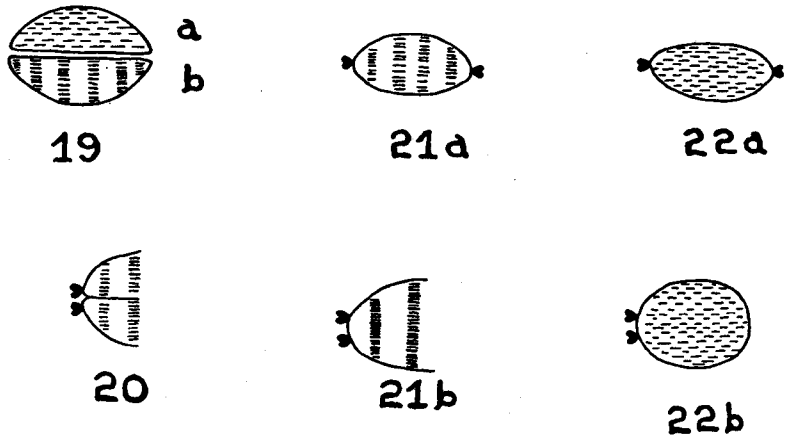
matic regions. According to work in progress, kinetochores have their independent period of sensitivity towards x-rays.

In Acrididae translocation at the kinetochore and joining of chromosomes with terminal kinetochores is frequent (King and Beams, 1938). In this group the same authors have described multiple chromosomes corresponding in type but not in make-up to the diplochromosomes of White. In the latter a "single" kinetochore carries two pairs of sister chromatids on either laterality. In the former, "decades and octades" may be formed, apparently by the association of non-homologous kinetochores. Geitler (1938*a*) considers the kinetochore in meiosis of *Paris* as double in structure, regardless of whether it functions thus or not. The behavior of the kinetochore does not influence the behavior of the chromatids. These may separate widely while the kinetochore behaves as if it were unsplit. The same author also does not believe that the threads connecting the kinetic bodies at early anaphase with the central achromatic body of the kinetochore are truly chromatic. Schrader (1935) has suggested that in the first division of *Protenor* there may be a broad or diffuse kinetochore over a large area of the chromosome, whereas in the second division this same chromosome reacts as though it had no kinetochore. Geitler (1938*a*) considers this viewpoint untenable, but it appears as a disagreement on definition rather than on fact. As long as nothing is known of the material basis involved in a kinetochore, morphological definitions may be made to fit any observed facts.

Thus if the kinetochore is defined as the region responding to the spindle mechanism at a given time, there is no objection to Schrader's statement. Thus even the akinetic fragments of Carlson (1938*a*, *b*) would show certain "kinetochoric" properties relating them to the spindle. Apparently these forces relating and synchronizing the kinetochores are highly labile and subject to compound conditions; otherwise it would be difficult to reconcile what Bleier (1938) and Metz (1938) have shown, that different or even externally similar chromosomes may move in opposite directions within the same half-spindle.

Recent work on abnormal division of the kinetochore has been published by Koller (1938) and Darlington (1939*a*). Darlington reports that in *Fritillaria kamtschatkensis* lagging meiotic chromosomes may divide transversely at the kinetochore. This is called misdivision. Also on hypothetical grounds Darlington supposes

that the kinetochore may break loose entirely and go to the pole alone. A transversely broken kinetochore may function normally as a terminal kinetochore, or it may give rise to an iso-chromosome by allowing one of the two chromatids on the same side at the time of the break to revolve through 180 degrees, thus making a new bibrachial chromosome with two identical arms, each having the



Figs. 19 to 22*b*: Tentative diagrams of "misdivision" of the central achromatic body of the kinetochore. It is assumed that transverse division is made possible by the arrangement of micellae in corresponding rows (Fig. 19*b*). A transversely divided central achromatic body is shown in Fig. 20. This may rearrange itself according to Figs. 21*a* or *b*. Internal reconstruction is achieved according to Figs. 22*a* or *b*. (See text p. 596.)

same gene loci but in relatively reversed order. This neoformation has previously been reported by Rhoades (1938*a*). Darlington speculates that the kinetochore is made up of a fibrous element consisting of units which he calls centrogenes, which would correspond to nemameres of my terminology, surrounded by a fluid coat which explodes at metaphase. If the centrogenes or nemameres have properly divided before the explosion, the division will be normal; otherwise it will be transverse. One might venture a different speculation not encumbered by the postulate of splitting time. In diagram 19*a* the micellae making up the central achromatic body are arranged in alternating rows, a condition in which normal division is supposed to occur. In diagram 19*b* the micellae are arranged in corresponding rows, thus allowing transverse division. It is suggested that the freshly and transversely broken central achromatic

body has the same ability to rejoin such free "sister" ends as chromatids or half-chromatids may have. This is illustrated in diagrams 20 to 22. Diagram 20 shows a central achromatic body with joining chromomeres and micellae in corresponding rows; a transverse break has occurred. In diagram 21*a* one of the broken halves has revolved and joined its original sister. In diagram 22*a* the joining chromomeres have multiplied and the micellae are again arranged alternatingly. In diagram 21*b* an alternate development from that shown in diagrams 21*a* and 22*a* is illustrated. Reconstruction has taken place by uniting the two achromatic half-bodies, diagram 21*b*, and rearranging the micellae into the alternating order (diagram 22*b*).

CHROMOMERES

Chromomeres are the smallest particles identifiable by their characteristic size and position in the chromosome thread between leptotene and pachytene and in salivary gland nuclei. (Def. Darlington, 1937, after Wilson, 1896.)

Chromomeres, as their name implies, take nuclear stains and serve as permanent markers on cytological chromosome maps. Since the term is morphological, its use has been extended to any stage of the nuclear cycle and is now used to apply in mitosis, in meiosis and in polytene stages of the nucleus. With this extension the term has lost much of its general genetic significance which some had hoped it might acquire. One might re-define the term: Chromomeres are constantly reproducable staining bodies or areas of chromonemata in certain stages and in certain organisms. Taking chromomeres in the original meaning of the term first, one must consider the pachytene thickening of corn chromosomes as typical examples. Heilborn (1939) has shown that to obtain such chromomeres, even in Belling's classical object, the lily, it is necessary to kill quickly with acetic acid. Thus one obtains a variety of reproducible chromomeres partly discoid in shape and ranging to the limit of resolution in thickness. Heitz (1935) remarks that chromomeres are evenly and linearly distributed over the length of the chromonema. The threads connecting them are called fibrillae. This evenness of distribution, which is not general, has allowed certain workers to interpret chromomeres as optical sections of successive helices of a coiled thread. This fallacy has been discussed by Kaufmann (1936).

From these true zygotene-pachytene chromomeres of meiotic prophase, certain heavily staining areas found in somatic metaphase chromosomes should be clearly distinguished. Each of those, according to Geitler (1938*c*), must correspond to several gyres of the helix, so that adjacent chromomeres of different gyres must be united. The large interstitial pieces signify a certain architecture of the chromosome not visible in pachytene. These latter bodies have recently been described by Kostoff (1938*a*). Ellenhorn (1937) considered the compound chromomeres observed at mitosis to give the most detailed morphological characteristic of somatic chromosomes.

Finally, the chromatic bands of salivary and other polytene chromosomes form a separate category of chromomeres. All chromomeres have been subject to speculation since the time when Belling believed to have seen a gene. Koltzoff (1938) has drawn instructive diagrams of how elementary chromomeres—the smallest visible units—may combine in small groups to form secondaries and these again tertiaries. The genonema at all times connects only single elementary chromomeres but this can not be seen, as nucleic acid, not an ingredient of the genes, may form a protective sheath around compound aggregations of chromomeres.

Painter (1939) suggests that ultimate chromomeres are arranged in a narrow spiral so that visible chromomeres are collective bodies with a spiral within them. Upon stretching, somatic synapsis occurs. Painter and Griffin have traced the salivary chromosome back to the four-strand stage. The spreading during growth of the polytene chromosomes is due to uncoiling of the ultimate chromomeres. As through multiplication the chromomeres become crowded, the chromatin is shifted to the free ends of the chromomere where it unites apparently into a transverse plate. Metz (1937*a, b, c*) cautions that in the polytene chromosomes chromonemata can not be seen individually in the later stages. The lines connecting successive granules are interphases between the achromatic droplets separating one disc from the next. Upon stretching, strands appear as artefacts. The smallest unit loss involves one chromatic disc and the layer of achromatic material on one side of it, but the units are probably not typically made up that way. Buck (1939*a*) has seen the banding of polytene chromosomes in the living and describes their optical cross section as round with a

dark margin and a uniformly granular interior without visible chromonemata.

Gates (1938) suggests that the classical work of Wenrich be redone with modern methods. Indeed, artificial stretching might reveal the compound nature of *Phrynotettix* chromomeres. Corey (1938) has described the heteropyknotic elements of Orthopteran chromosomes as consisting of polar granules which may fuse to form polar aggregates. These proximal granules persist through interphase, resembling heteropyknotic regions of plant chromosomes. Still a different type of chromomere mentioned earlier is represented in the distal end of chromosomes which may be called telomere (*cf.* Raffel, 1939). Chromocenters may be classified as compound chromosomic structures. Thus Marquardt (1937*b*) described the proximal regions of *Oenothera* chromosomes which are present in the premeiotic interphase nucleus as heterochromatic chromocenters located on the periphery of the nucleus.

There exists a graded series between forms with large chromosomes having certain heterochromatic areas or chromomeres which survive interphase without apparent change, and forms with small chromosomes showing prochromosomes during interphase. Such prochromosomes have recently been shown by Jouvenelle (1939). Raghavan (1938) described the behavior of such prochromosomes in Capparidaceae. Pairing commences at the kinetochores but a typical leptotene stage was not observed. Pachytene is the first obvious prophase stage of meiosis. In *Polanisia* a part of the chromatin may be accumulated on the surface of the nucleolus whose surface consequently presents darkly stained patches from late telophase to late prophase.

MATRIX, NUCLEOLUS, HETEROCHROMATIN

Chromosomes during mitosis ordinarily do not reveal the coiled chromonemata which they contain. This is due to the presence of a considerable amount of matrix (Sharp), kalymma (Heitz), hyalonema (Kuwada) or Hüllsubstanz, synonymous terms for a morphological entity, at present insufficiently defined in terms of function or chemical constitution. The matrix being a definite attribute of chromosomes in mitosis and meiosis, it might appear safe to assume that the matrical material is of near genic constitution and may furnish the building stones for genic multiplication and metabolism. Since the size of the gene-threads proper is below

that of optical resolution, chromonemata are "encased gene-threads," and if the encasing material is called matrix, it must be decided arbitrarily where the matrical material as seen in fixed preparations was originally located in the living cells.

In the morphological sense any or all of the material of a chromosome, excluding the chromonemata and the kinetochore, deserves to be described as matrix. The justification of the term has been variously discussed by Darlington (1935*b*), Heitz (1935) and by Nebel (1932), but at present one may look upon the matrix of a chromosome as a distinct substance which may vary considerably according to treatment and stage (*cf.* Schneider, 1938).

Thus one has, on the one hand, the matrix as the aggregation of substance surrounding the gene-threads, which one might speak of as the matrix in its centripetal function. On the other hand, there may be a matrical element which one may describe as having a peripheral function. Thus certain investigators have seen the chromosome or the chromonemata to be surrounded by pellicles. These pellicles are considered as of a matrical nature. As will be discussed in the chapter on coiling, matrical pellicles may fulfill important mechanical functions and may, hypothetically at least, be present in multiples, a chromosome pellicle enclosing two chromatid pellicles, for instance. If this latter viewpoint is correct, one may further postulate a cortical matrix substance and an internal matrical substance. The former may be derived from the latter and both may stem from the karyolymph of interphase and prophase. Geitler (1938*a*) considers the possibility that the axial cavity of the chromonema—the space around which the helix of the chromonema is wound—may be filled with matrical substance. The presence of such an axial space is, however, far from proved. In principle, Geitler (1938*a*), Straub (1938), Darlington (1937*a*) and Nebel (1937*b*) agree that in the living most matrical material in a metaphase chromosome is apportioned to specific chromonemata. The matrices of telophasic chromosomes contribute to what appears to be karyolymph, and from this in turn a certain substance is segregated out to build the nucleoli. Geitler (1938*a*) has suggested that the matrical concentration may decrease towards the periphery of a chromonema. "The helix gradually intergrades with the matrix. The matrix facing the exterior is deemed less viscous than the matrix of the interior." This was previously stated by Nebel (1932, *253*). Matrical chromatin is commonly be-

lieved to serve as an insulating sheath protecting the genes. With this in mind, Cooper (1938) suggests that synapsis may be governed by matrical distribution in so far as at meiosis the matrical envelope may be incomplete, allowing of and limiting pairing to a distinct surface of the chromosome. Northen (1937) suggested that each chromosome exercises control over a distinct amount of matrical karyolymph, which is in agreement with the existence of karyomeres in the resting nucleus. Goldschmidt (1937) found the weak races in *Lymantria* to have larger chromosomes than the strong races. Either the cytoplasmic condition called strong and weak F, controls the size of the chromosomes, or the established correlation has no statistical significance. The amount of matrix may be influenced by nutrition and metabolism.

According to its reaction towards basic dyes, the chromatin of the matrix may be classified as eu- or normal, and hetero- or different chromatin. Probably the distinction is due to differences in the underlying gene-string. Heterochromatin does not become invisible during interphase (Heitz, 1936), but remains condensed and stainable, and during prophase heterochromatic chromosome regions may be premature in development. The difference between the two chromatins is intergrading (Poulson, 1938; Poulson and Metz, 1938). According to Straub's review (1938*b*), heterochromatic chromosomes or segments may not properly unwind their spirals, and they may show fewer chiasmata during synapsis. Geitler (1938*a*) considers heterochromatic regions less active metabolically. Heterochromatin may be mere ballast or have certain trophic value. Heterochromatic chromosomes are considered to have fewer genes than euchromatic ones. Heterochromatic areas are apt to break under radiation as euchromatic ones would (Kaufmann and Demerec, 1937). This point is still under dispute. Hett (1937) has shown how nuclear and nucleolar material may be ejected into the cytoplasm, possibly aiding secretion. Heterochromatin tends to be located near the kinetochore (Kostoff, 1938*c*) in *Nicotiana*-crosses and in *Oenothera* (Straub, 1938*b*). Lorbeer (1938) was able to modify sex characters located in the heterochromatic x of liverworts, changing femaleness into maleness and synoecousness in one species, and from femaleness to maleness only, in another. It is not excluded that the primary changes involved were purely quantitative.

With regard to work on the nucleolus it must be said that the

understanding of this problem has not progressed far during the last fifty years. Wilson (1928) recognized plasmosomes, karyosomes, chromosome nucleoli, karyospheres and amphinucleoli. As morphological types none of these distinctions has become invalid as yet. Recent work has further emphasized the complexity of the problem so that it is necessary to avoid all generalizations. All that can be said is that certain globular aggregations of materials occur in the nuclei of plants and animals which may be summarily called nucleoli.

All other distinctions of these bodies must at present still be limited to systematic or functional groups, and the occurrence of exceptions to any rules must be allowed for. Geitler (1938*a*) discusses the existing knowledge on the nucleolus comprehensively. Thus it is not yet decided whether the loci at which, according to Heitz, nucleoli condense are truly chromatic or not. As these regions are highly attenuated, this will be hard to decide by the Feulgen method. One must, as Sharp (1934) and Geitler (1938*a*) have done, object to the conclusion that there is any direct relation between chromosomal and nucleolar materials, although certain cases suggest this. Occasionally the nucleoli will not condense on the existing constrictions.

Geitler (1938*a*) has also called attention to the cases in angiosperms and Protista where nucleoli survive mitosis. In *Spirogyra* no nucleolus-organizing regions are known, and nucleolar material may be transported by anaphasic chromosomes which are thus increased in diameter. In *Amoeba dubia* interphasic nuclei contain no nucleoli, but during prophase achromatic bodies appear which are passively transported during anaphase and disappear again during telophase.

Much work has been done on the nucleolar and heterochromatic properties of polytene chromosomes of *Diptera,* but this field has become too large for extensive discussion here. Frolova (1937; 1938) has shown how the chromocenter is differently composed in various species of *Drosophila,* being made up in *D. funebris, e.g.,* from the heterochromatin of the x. In *D. melanogaster* various parts of the chromocenter belong to separate chromosomes. Maising and Sokolov (1938) have recorded a case demonstrating the mutual attraction at different levels of the same chromosome and mutual attraction of distinct chromocentric regions into a common center. In *Sciara* certain regions of the polytene chromosomes

appear "puffed" and are considered nucleolar in nature (Poulson and Metz, 1938), whereas in *Drosophila melanogaster,* according to Kaufmann (1938), nucleolus-organizing regions exist in the heterochromatin of the sex chromosomes.

Nucleoli of non-polytene nuclei are still the object of many detailed studies, since their behavior is variable in many ways. Heitz (1935) established the general law that nucleoli become condensed on distinct chromosomes on special threads which carry trabants. This law is generally true, but modifications of it exist. McClintock (1934) apparently described a special case when she observed nucleoli to be formed directly from the matrices of particular chromosomes in corn quartets. Usually the transformation of matrical material into nucleolar material is indirect. In case there is no nucleolus-organizing body, the matrix material from the various chromosomes accumulates in droplets in the nucleus (Sharp, 1934, *120*). Gates (1938) tentatively considers matrix and nucleolus directly related. In *Trillium kamtschaticum,* according to Matsuura (1938*a*), both distal ends of chromosome A and the distal end of the short arm of E are nucleolus forming. Under special conditions each chromosome may become nucleolus-forming. In meiosis of *Fritillaria* (Frankel, 1937) small globules appear in early telophase of both divisions located at the distal ends of the chromosomes. They are subsequently dispersed over the body of the cell. Ito (1938) showed the extrusion of nucleolar material into the cytoplasm in a teleost. Secondary nucleoli arising from a primary nucleolus were shown by Eichhorn (1937) in *Carica.* These remain during division and are dragged to the poles with the chromosomes. In telophase they may grow and fuse again.

Pätau (1937) described a special achromatic fibre which in *Collozoum* connects the satellite with its chromosome. This is incased by the nucleolus which during prophase carries two more deeply staining polar caps. Also the achromatic fibril is double in prophase, and the nucleolus divides in metaphase. Okuno (1937) describes the nucleolus of *Lobelia* as dividing into two, one of which is connected with the distal end of a distinct chromosome, the other remaining unattached. Both nucleoli disappear gradually toward metaphase. In *Sauromatum* (Geitler, 1938*c*) there are two satellited chromosomes with nucleoli of unequal size. One of these possesses a detachable bud. Woods (1937*b*) has shown that in *Tulipa,* comprising much polyploidy, the law of Heitz may not

apply rigidly. The relationships of number and size of nucleoli are not simple. Nucleolar fusion is frequent. In the variety, Mrs. Moon, no organized nucleoli appear in the microspore and are hence considered non-essential. Yuasa (1938) believes that in Myxomycetes the blepharoplast originates from the nucleus in connection with the nucleolus. Bhatia (1938) has compared nucleolus sizes in certain wheats and concludes that the total volume of one compound nucleolus is greater than that of many single ones. Darlington and Thomas (1937) find in *Lolium* that nucleoli may be developed even while the chromosomes are still condensing. Lesley (1938) discovered three races in tomatoes differing in size of nucleolus. The satellite volume in the races is as 1:2:4. Nucleolus volume increases with the length of *"A,"* the attachment thread of the satellite. However, Resende (1939) reported a case in which the attachment thread of the satellite increased in length before telophase. Thus he believes that the extent of this attachment thread varies independently of the size of the nucleolus to be formed on it. Wolcott (1939) described different modes of attachment of nucleoli; thus the end of the chromosome may be flush with the surface of the nucleolus or attenuated. Geitler (1938*a*) believes that the nucleolus-organizing regions may have been gradually developed from less differentiated forms. Sato (1938) has shown Heitz's law to hold well in the Amaryllidaceae. Kuhn (1938) has settled the dispute on trabants in stock and maintains that *"let"* plants have two trabants as normal plants. The satellites are of the heterochromatic type, and it is not proved that the factor for double flowers is located in the trabant chromosomes. Thus nucleoli are as yet not understood. Their consistent behavior makes them important cytogenetic landmarks. If the behavior of nucleoli in certain forms diverges from that of others, one is as yet not able to assign any functional significance to such divergence.

POLARIZED LIGHT

Through the endeavors of Schmidt (1937), Frey-Wyssling (1938) and others, the tool of polarized light has been made very much more accessible to biologists than it previously had been. According to Schmidt, chromatin consists of stretched negatively uniaxial doubly refractive "submicrons" which under desiccation become arranged in parallel. Chromatin, then, may show positive "form" double refraction or negative "eigen" birefringence, accord-

ing to the degree of desiccation. The one may compensate the other. Under heavy swelling double refraction may be lost. Under heavy desiccation the negative double refraction may be lost. The negative double refraction of chromatin is due to its nucleic acid component. These findings have been confirmed by Frey-Wyssling (1938) and by Caspersson (1937) and applied to the structure of the polytene chromosome by Signer, Caspersson and Hammersten (1938). Thus it may be considered a safe assumption that the nucleic acid chains are themselves negatively birefringent and probably arranged parallel to the main axis of the chromonema.

On this basis a series of papers has been published by Kuwada (1936; 1937; 1938), Kuwada and Nakamura (1934; 1935; 1938), and by Nakamura (1937). It has been assumed by these authors that the evidence fully confirms the general concept of helication in *Tradescantia*. During somatic anaphase the coils are at right angles to the axis of the chromosome, and double refraction is positive with regard to the axis of the chromosome. During the first meiotic anaphase the axis of double refraction is at right angles to the main axis of the major helix, which is in agreement with the double coiled condition of this stage. During interphases, results are not so clearly defined, but the axis of double refraction appears to be at right angles to the major diameter of the nucleus, and it is concluded that this is to be expected on the grounds of straight chromonemata running lengthwise in the nucleus. I had compiled a list of questions raised by the Japanese workers when the work of Becker (1938) came to hand, giving these questions even more concrete form. Becker has shown that one can obtain results with polarized light mostly in accordance with what the direct image also shows.

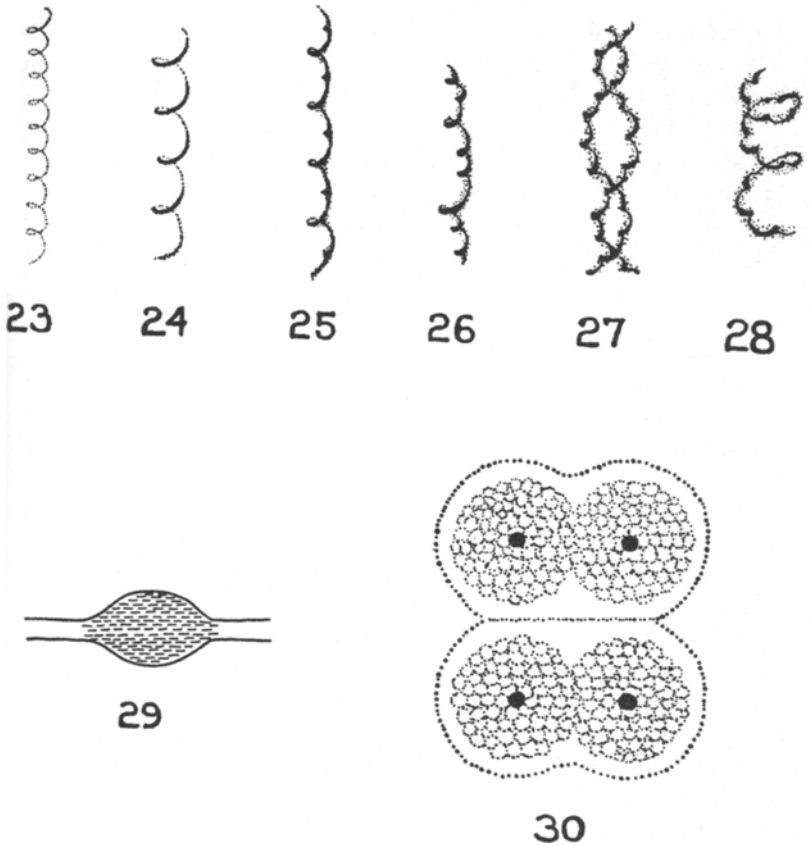
After block-fixation the axis of birefringence in anaphase chromosomes is at right angles to the axis of the chromosome. After fixation with acetic acid, rendering coils visible, the optical axis is partly changed. Becker was not able to confirm the Japanese results on chromosomes of meiosis, although he used two species of *Tradescantia* and *Gingko biloba*. Becker believes that the optical results are due to chromatin encasing the spiral, perhaps not the spiral proper. This is also considered by Nakamura (1937). Probably acetic acid increases helication, ammonia decreases it. Perhaps the following points want further investigation: Would a stretched chromatin thread show a reversed birefringence compared

with a coiled thread? If it is generally conceded that chromonemata in the resting nuclei of *Tradescantia* are far from straight and parallel throughout, then, are the results of the Japanese, that resting nuclei show negative birefringence, due to double coiling as it may exist in spiral prophase, or are they due to a general oriented distribution of karyolymph? How would one explain a reversal in the direction of the axis of birefringence between the anaphase of the second meiotic division and the first gametophytic division, if not on the basis of fixation? If this admission is made, I conclude that the method of polarized light, as the Japanese used it, confirmed what had been seen previously but could not be used for conclusions beyond this limit. Confirmation of the results of one method by the use of another is worthwhile, and the experience of Nakamura that the chromonema proper is not birefringent deserves further attention. If this be literally true, a method would be on hand to distinguish between matrix and chromonema. Would it not appear more likely, however, that the boiling water which was employed destroyed the birefringence of the chromonema, rather than that the chromonema never possessed this quality at all? Even leptotene chromonemata appear to carry nucleic acid at least in the chromomeres; hence these regions should be negatively birefringent. In diagram 29 a chromomere is shown in which the nucleic acid micellae are arranged parallel to the thread axis; this would result in negative birefringence. A study of liliaceous leptotene threads with polarized light should form the natural starting point for any investigation on chromosomes because no complex structure can be adequately understood unless its main constituent is properly investigated in its supposedly simplest state.

COILING

To one not engaged in the study of chromosome coiling this field must appear somewhat bewildering. There are standard coils, minor coils, major coils, relational coils, relic coils, molecular coils, twists, super coils, hysteresis and perhaps several more terms, some of which actually appear necessary at present. To take the chromosomes of any liliaceous plant, for an example, and to illustrate the terminology with a few simple diagrams will, it is hoped, not be amiss.

In somatic metaphase the chromonemata of a chromosome are helicated, and this helix is for each and every species its respective



Figs. 23 to 28: Coiling of chromonemata. Fig. 23: The standard coil of somatic metaphase. Fig. 24: The relic coil of early somatic prophase. Fig. 25: Relic and incipient standard coil of the next metaphase. Fig. 26: The new standard coil attains standard dimensions, the relic coil is nearly lost. Fig. 27: Relational coiling between chromatids. Fig. 28: Major and minor spiral of meiosis. (See text p. 606–608.) Fig. 29: Tentative diagram of an ultimate chromomere which is considered a local aggregation of chromatin on the gene string. (See text p. 606.)

Fig. 30: Cross section through a somatic telophase chromosome. Each chromatid contains two gene threads (black) which are surrounded by "near genic" matrical micellae shown in cross section. The behavior of these latter units is believed to be governed by the central gene thread which in turn may be protected nourished and mechanically strengthened by the matrical units. The matrical micellae are surrounded by a certain amount of karyolymph which forms the interface between the chromosome and the cytoplasm. (See text p. 614–615.)

standard coil. One may call the measured diameter of this coil *1* for reference (diagram 23). This standard coil persists through telophase and, according to some, through interphase in certain species. In early prophase the gyres of this standard coil are widened and finally lost. During this interval this late standard coil is called a relic coil (diagrams 24 to 26). While the chromonemata are in the relic coiled state a new standard coil begins to develop (diagram 25) which toward the next metaphase again reaches standard dimensions (diagram 26). Coils drawn so far represent a chromonema in a chromatid. During any stage of the somatic cycle chromatids may be wrapped around one another. This entanglement is referred to as relational coiling. Relational coiling may be tight, as in an electric flex (diagram 27), or quite loose. It may be in the same direction throughout, or it may change direction. It is more prominent in some species than in others but probably never quite absent. So far for mitosis. In meiosis the first division is in many liliaceous species characterized by very much larger helices called major coils. Their diameter may be *2* or *3* as compared with *1* of the standard coil. Occasionally and especially if a slightly alkaline pretreatment is used, another much smaller coil is seen running over the gyres of the major coil. This is called the minor coil. The gyres of the latter are < 1 (see diagram 28). The behavior of the major and the minor coil varies between species after the first division. In *Tradescantia,* however, the minor coil grows before the second meiotic metaphase and becomes a standard coil again at the second metaphase.

The molecular coil is a speculative assumption, according to which the molecules of the gene threads are joined at an angle. In effect such a condition would be the same as an imposed twist. Other terms, such as super coils, may be discarded.

The discussion on coiling centers around the following points: What are the observed facts about coiling? What do these facts imply, and can we think of a model that could satisfy the implications?

Straub (1938*b*) considers coiling a vital phenomenon. Sister chromonemata coil in the same direction. The relational coils appear to be created by formation or resolution of the standard coils. According to Straub, we do not know just how this comes about. Geitler (1938*a*) would like to call the major coils "Eigen-spirals," meaning that they comprise entire chromosomes, and in this there

is little dispute; but it would not be fitting to drop the term minor spiral, since the minor spiral is actually smaller than the standard spiral (diagram 28). That external conditions may modify the degree of coiling has been recognized by Geitler (1938*a*), Huskins and Wilson (1938), Sax (1937*b*) and others as discussed under methods.

The direction of coiling has recently been reinvestigated by Huskins and Wilson (1938) and by Matsuura (1938*a, b, c*). The former found that in meiosis of *Trillium* the direction of coiling is random on each side of the kinetochore. Chromatids which will be mates at first anaphase do not necessarily coil in parallel. There will be one half as many changes in direction as there are chromatid attachments. The remaining number of reversals is proportional to the total number of gyres and hence must be a function of gyre-frequency or of chromonema length. However, the high number of reversals in direction of coiling in asynaptic material suggests that the importance of chiasmata has been overestimated. White has, according to Huskins and Wilson (1938), demonstrated reversals of coiling in mitotic chromosomes of grasshoppers. If coiling of chromatids is initiated before crossing-over takes place, one might think of small interstitial reversals in coiling being resolved during early diplotene, which would account for the observed connection between chiasmata and reversals as a secondary phenomenon. Matsuura (1937*a, b, c;* 1938*a, b, c*) analyzed with outstanding diligence the direction of coiling in *Trillium kamtschaticum.* His factual evidence coincides fully with that of Huskins and his collaborators. Chiasmata have no relation to reversals of coiling, most of the latter being independent of one another and fortuitous. Unfortunately the neo-two plane theory of crossing-over of Matsuura is based on the erroneous assumption that an arrangement of chromatids and kinetochores at the time of crossing-over can be inferred from the configuration observed at prometaphase. Since the direction of coiling in somatic chromosomes can not be studied accurately so far for lack of adequate resolution, one can only estimate the number of reversals of coiling in such chromosomes or chromatids. These reversals may vary between species, but in *Tradescantia* I would estimate not more than one to occur per five gyres of the helix. Certainly reversals are much less frequent than gyre number.

Upcott (1938) studied the relic coils in *Hyacinthus,* which one

assumes reflect the direction of the previous standard coil. She compared this with the relational coiling existing at the same time which may be linked to the direction of the new standard coil just being initiated. The conclusion is that the directions of coiling in successive mitoses are indeterminate and characteristic of individual mitoses but not of individual chromosomes.

Takamine (1937) observed the persistence of the minor coil through interkinesis in *Hosta* which thus is typically similar to *Tradescantia.* Shimakura (1937) relates the visibility of half-chromatids in *Trillium* during first meiotic anaphase, and this material was mounted in saccharose so that the cells were "fresh" rather than fixed. Shinke (1937) studied the reversible changes of living plant nuclei in the resting stage. His findings are not utterly original but represent a modern interpretation. In the living any plant cell nucleus may be homogeneous but is not necessarily so. The nucleolus is usually visible; on treatment with hypertonic media nuclei become increasingly chromonematic. Chromocenters under the influence of ammonium chloride appeared to unravel with only small granules remaining. The chromonemata revealed in interphase of living cells by Shinke were thus coiled, not straight, at least in the plants with massive nuclei. Pätau's (1937) work on *Collozoum* deals with the same subject, and here also coils are visible in telophase and interphase, but there are also chromomeres which may contribute to the granular aspect of the interphase nucleus. This is probably true of all interphasic nuclei. Jeffrey (1937*a, b*) and Jeffrey and Haertl (1938) have described somatic coiling in *Trillium.* The chromatids here in anaphase are arranged as in a loosely coiled electric flex.

Koller (1938), working on the golden hamster, pictures the mitotic coiling of prophase to start with many tight gyres which become progressively wider and fewer towards metaphase. This is not in general agreement with plant chromosomes where the early standard coils appear to be of smaller gyre but not much, if any, more numerous than the final standard gyres. Cleveland (1938) shows how in *Spirotychonympha* a somatic coil may reverse its direction at any point in a chromosome and how the coils of daughter chromosomes may be directed alike or differently. Bauer (1938) emphasizes how each chromatid has its independent coil and how in certain protozoa metaphase occurs before the coils are properly developed. Maximum spiralization here occurs at the end of anaphase.

Knots in chromosomes have been postulated and reported by Husted (1937; 1939) and by Metz (1937*b*). The knots observed by Metz in polytene chromosomes apparently are formed by movements of free ends, whereas those of Husted observed in *Paris* and *Pancratium* are considered the result of breakage. Broken ends will reunite after another portion of the chromosome, a loop, for example, has been pushed through the broken region, or a structural change has occurred. Knots in Husted's material may become untied before metaphase without resulting abnormalities.

The phenomenon of chromosome coiling has attracted a great deal of speculative comment. One group of discussions centers around the mechanism of coiling while another deals with the role of coiling in chromosome mechanics.

The mechanism of coiling has been the subject of a recent paper by Nebel (1939) which may be summarized briefly as follows: Coiling may be due to expansion of the chromonema in a confined cylindrical space. If one takes an empty milk bottle and forces a rubber hose into it, a coil of this type will be formed. Reversals will take place if the ends of the hose are prevented from rotating. This sort of scheme may be called the matrical interpretation since the matrix is thought to form a rigid pellicle within which the chromonemata are forced to coil. With minor modifications this scheme is adopted by Huskins and Smith (1935), Kuwada (1937), Sax (1936) and Wilson and Huskins (1939).

The main weakness of this theory is that the pellicle postulated has not been commonly observed, and that it is not generally admitted that the chromonema during maximum coiling is longer than during early prophase, when the thread is supposedly more relaxed. Darlington (1935) postulated an internal force to explain coiling and called this the molecular coil. According to this, the molecules of the gene-thread are asymmetrical so as to create a twist in the thread which is untwisted in space through the establishment of a coil. As the picture of an internal active mechanism of coiling appealed to me more than the pellicular mechanism, a model was made to justify the molecular theory and obviate its strongest shortcoming, which is its inability to explain why or how coiling is redetermined at random at each successive division and probably likewise in meiosis before the meiotic coils are established.

Instead of using the term molecules, the ultimate mechanical units of a gene-thread have been called nemameres. If nemameres

of half-chromatids separate just before the new standard coil commences to be formed, it is suggested that during separation there is a rotational repulsion of individual units in such a way that both the "new" and the "old" thread become twisted in space, dextrorsely or sinistrorsely. Starting at various points along the thread, the reaction of separation spreads from one or a few initial points in the chromosome over its entire length. Thus in somatic mitosis during prophase, the two half-chromatids in a chromatid will coil in parallel, whereas sister chromatids may coil independently. The scheme can be applied to meiosis where two events of separation must be assumed, the one determining the direction of the major, the other that of the minor coil. One can imagine that two threads previously separated—the chromatids as they exist before leptotene —may be reunited during leptotene, and that the renewed separation of such sister threads allows of reestablishing a new coil.

One may ask why such an elaborate scheme is needed to describe coiling and gene multiplication. The reason for this is as follows: Extensive work on ring chromosomes (McClintock, 1938*b*) has shown that the division plane in a chromosome is ordinarily orthotropic which means that a ring chromosome may divide in such a way as to give rise to two identical rings. Occasionally, it is true, non-disjunctional figures arise such as interlocked rings or a continuous double sized ring. These phenomena may come about by crossing-over between sister chromatids or by a twist in the divisional plane. This presents the mechanical problem: The gene-threads, so as to separate, must not be twisted around each other. Yet if the threads multiply when they are not coiled, how do they avoid entanglement during spiralization? According to the pellicular theory, one must assume that multiplication occurs while the threads are straight and that successive coiling will not cause serious tangling or that multiplication occurs in the coiled state orthotropically, that is, in one direction in space. According to the nemameric theory, threads twist internally as a result of separation but the resultant coil untwists the thread so that there are not only no internal twists in the coiled state but also no entanglements of sister threads.

The phenomenon of relational coiling comes into this discussion only indirectly. Relational coiling refers to the fact that sister chromatids in mitosis are wrapped around one another. It has been variously suggested that relational coiling is caused by residual

friction between adjacent chromatids, while their constituent threads are twisting or untwisting. On the grounds of the pellicular theory, there should not be any such residual external forces, so that relational coiling in itself argues against that theory.

There are several other suggestions which bear on the mechanics of coiling: Cooper (1938) has pointed out that if a number of coiled chromonemata are closely appressed to one another, the increase in number of size of the chromonemata will tend to uncoil the original helix.

Does the major or the minor coil originate first in meiosis? According to Sax, quoted after Straub (1938*b*), and according to Darlington (1937*b*), the minor coil originates first and is then secondarily coiled by the major coil. According to Kuwada (after Kaufmann, 1936), the major coil comes first. My own interpretation is that the major coil of meiosis is homologous with a relic coil of mitosis. Thus a new coil arises after leptotene and transgresses standard dimensions during diplotene. It is held in relic dimensions during first meiotic division and in some species even longer. The minor coil on this interpretation comes after the major coil just as the new standard follows the relic coil. This natural process may be slowed or speeded so that one may have a standard coil at first division as a result of straightening of the major coil or because a major coil was never formed. This analogy is implied also by the work of Koller (1938) who found that the differential segment of a hamster sex chromosome at meiotic metaphase had a minor, relic and an interchromatid relational coil.

Huskins (1937) challenged the Japanese workers on the reality of the minor coil and its optical relation to the presence of half-chromatids in meiosis. In two papers of Kuwada (1938) and Kuwada and Nakamura (1938), both points were answered, and now the early minor coil may be looked upon as a corrugation if one so desires and if the presence of half-chromatids is admitted.

Kuwada, Shinke and Oura (1938) have suggested that mechanical drawing-out of a spiral would lead to a twist of the thread thus drawn-out, whereas chemical drawing-out would have no such result. Just what is meant is not clear. The drawing-out in the latter instance might occur slowly so that the thread would have time to untwist by rotation. Süssenguth (1938) has suggested that the attraction of successive carbon and amino groups in the gene string causes spiralling. Away from the isoeletric point the

spiral is relaxed. Any general theory of coiling will have to take into account the fact that coils of the chromonema follow one another as successive series of sound waves issuing from a central source, the gyres becoming progressively larger until they are straight again. However, this process may become arrested or partly reversed at any stage and may be governed by or subject to changes in internal pH.

In the general theory of crossing-over, both Sax and Darlington have employed the stress of relational coiling as one of the forces which enter into the process. So far an attempt by Husted (1938) to compare somatic relational coiling with meiotic chiasma frequency has not shown a distinct correlation.

GENIC MULTIPLICATION

What a gene is we do not know. How genes multiply we do not know. Any discussion of genic multiplication, if it is not speculation, thus relates to the time of splitting of the chromonema. It is an unsafe assumption that a chromonema splits because the genes within it have multiplied; it is, however, the assumption closest at hand, if one does not want to assume that gene multiplication and the visible division of the chromonema occur simultaneously. According to Frolova (1938), Gates (1938), Marshak (1937) and Straub (1938*b*), division of threads occurs in prophase. According to Koltzoff (1938), threads split during the period of maximum length. Most other investigators place multiplication in interphase (Berger, 1938*a, b, c;* Carlson, 1938*a, b*; Geitler, 1937; Goodspeed and Uber, 1939; Levan, 1939*b, c;* Kaufmann, 1936, who quotes Sax, Huskins and Kuwada). Genic multiplication should lead to splitting of chromonemata during maximum elongation in earliest prophase, but genic multiplication itself would thus fall into late interphase. If a gene-thread proper is no more than 20 mμ in diameter (Waddington, 1939), it would appear easiest to assume (*cf.* Snell, 1938) that it is surrounded by a number of near genic-threads which, however, must take their cue from the central master thread. The possibility of such auxiliary gene-threads is emphasized by the difficulty involved in ascribing to a single monomolecular filament all the mechanical properties of attraction, repulsion, coherence and rigidity, let alone the genetical properties associated with the chromonema. Thus one arrives at the concept of a central gene-filament supported by a number of nearly identical

but dependent auxiliary gene-threads which in turn carry the nucleic acids and other matrical materials. This is illustrated in diagram 30 showing the cross section of what is believed an interphase chromosome. The term nemamere (see above) would then cover many molecules at a given level of the chromonema but perhaps only a single gene. The nemamere is different from the chromomere, the former having primarily functional and mechanical significance and thus not being limited to morphological appearance or a specific chromatin content.

It is suggested that a nemamere does not divide until the gene or genes which it contains divide and that thus division of genes proper is concomitant with the division of the visible chromonema.

There may be some regulatory mechanism between separate sister genes of the same chromosome, but of what nature this regulation is one does not know.

For the study of chromonemata more attention should perhaps be given to the protozoa (Cleveland, 1938). *Spirotrichonympha*, as drawn by Cleveland, shows chromonemata perhaps clearer than are found even in *Trillium*. Also the use of chemical treatments such as colchicine has not been exhaustively employed in this connection (Levan, 1939*b*). It may soon be possible to induce the formation of diplo- and polytene chromosomes at will (Levan, 1939*c*). If this be possible, many problems of gene mechanics would become more accessible. Further work on the unravelling of compound chromomeres (Kostoff, 1938; Painter, 1939) is also of fundamental importance. It is to be hoped that thus the gap between the biological interpretation and the chemical facts (*e.g.*, Caspersson) and physical findings (*e.g.*, Astbury) may eventually be bridged.

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