CHROMOSOME STUDIES ON *GASTERIA*
III. CHROMOSOME STRUCTURE DURING MICROSPOROGENESIS AND THE POSTMEIOTIC MITOSIS[1]

WM. RANDOLPH TAYLOR

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INTRODUCTION

The writer began regular collections for a study of chromosome form in *Gasteria* in 1922 at the University of Pennsylvania, following casual earlier observations indicating that this genus was suitable for careful cytological work. The study of chromosome form through the various developmental stages came to tolerably satisfactory completion and was published (1924, 1925, 1929). As it progressed, evidences of internal structural differentiation became apparent, and a preliminary note was published calling attention to one aspect of this (1922). Accumulating material of *Gasteria* and of other plants studied in the writer's laboratory soon showed the inadequacy of this preliminary interpretation, so that a redescription of the conditions was undertaken. The structure of the chromosomes being inseparable from the changes occurring during the meiotic phases, it was necessary to secure material for a description of meiosis, which task has, from interference by other work, been but slowly accomplished. Only the comparative adequacy of the available material in character and quantity, and its preparation by distinctly improved methods, justify the publication of another description of meiosis.

It is not worth while to analyze here the various views propounded regarding chromosome structure. The literature has been growing rapidly, and has been reviewed several times. It is impracticable to separate the literature upon chromomeres and chromonemata from that dealing with synapsis and the chromosome tetrad, with both of which this present paper is also concerned. The publications of Kaufmann (1926*a*, *b*) on *Tradescantia* and *Podophyllum* report the first studies in which the writer had a direct interest, and fairly review the earlier literature. A double spiral structure is described in somatic chromosomes, and a history contrasting with that offered by Martens (1922 *et seq.*). A spiral chromonema was also found in meiotic chromosomes. Spiral structures are next reported by Kuwada (1927) also in *Tradescantia*, who finds mechanical difficulties in separating

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the double spiral present. As will be seen by the account to follow, no such difficulty arises in *Gasteria*. Kuwada also discounts Kaufmann's description of double anaphase spirals. Disregarding theoretical interpretations offered, the writer can confirm Kaufmann's published observations from inspection of his beautifully prepared material, and is confident that optical illusions have not significantly contributed to the image recorded. Sakamura (1927) experimentally confirms, principally upon *Tradescantia*, the normal nature of this spiral structure in chromosomes, and shows under what conditions it may be demonstrated. Maeda (1928, 1930) in *Lathyrus* and *Vicia* also finds spiral structures. It appears that in *Lathyrus* the spiral form is clearly retained through interkinesis. Newton (1927) working with *Tulipa* gives a most acceptable account of meiotic prophases from the standpoint of synapsis and the development of the chromosome tetrad, but he recognizes the spiral elements only in the second prophases, at which time, though clear, they are comparatively uninteresting. A posthumous paper with Darlington (1929) also concerns itself with synapsis and the formation of tetrads or their equivalents. Babcock and Clausen (1929) report spiral chromonemata in *Crepis* and relate these to the tetrad. They record a contraction of the prophase threads to form the spiral chromonemata before diakinesis, an observation of interest since this period is obscure in *Gasteria*. Shinke (1930) surveys the list of plants showing spiral structure, making several additions, generalizes somewhat respecting the behavior of the threads through the meiotic prophases, and offers good evidence for the double nature of the thread in *Tradescantia*. He finds spiral structures in the chromosomes of the first pollen grain divisions of three genera. In vegetative cells a similar spiral structure has been reported by Kaufmann (1926*a*) and more recently by Sharp (1929), who discusses the literature and gives a very complete account of conditions in *Allium*, *Trillium*, *Vicia*, and *Podophyllum*. Similar structures and development occur in *Gasteria*, but the account of the writer's observations on that score are not ready for presentation.

Contrasted with the spiral chromonematal accounts of mitosis and meiosis are those based on chromomeres as exemplified by Belling (1928) and that involving the longitudinal cleaving of relatively condensed chromosomes with the consequent cutting of any transverse or spiral structures as represented by Martens (1922, 1925, 1929). Many of the structures which they found appear in the preparations of the writer, and the differences in interpretation will be sufficiently evident from a comparison of the reports.

First Maturation Division

I. Prophases

In *Gasteria* the resting period between the last archesporial division and the first meiotic prophase is a long one, during which there is considerable increase by growth in the bulk of the cells. Consequently there is little or

probably no chance of confusing telophasic stages of the last archesporial division with the prophases more properly under observation. In cells which have reached full growth the nuclei are about 12.2 μ [2] in diameter, nearly round, generally with one nucleolus, although up to a third of them have one or two additional nucleoli. The nucleus shows a uniform structure of dense, smoothly coagulated karyolymph, in which are embedded very many small granules of the order of 0.2–0.3 μ diameter, evenly distributed throughout the mass. These granules, while darker than the supporting matrix, do not take on a sharp chromatin stain. No definite connecting system is recognizable between them.

With advancing orientation of the nuclear contents for division the granularity becomes progressively emphasized, because of the appearance of elements which are able to hold the chromatin stains very strongly. The smallest of them are no larger than the granules seen during rest, but gradations to larger elements 0.5–1.0 μ in diameter are common. There is here also, even in the cells which have received most directly the action of the osmic acid, very little evidence of orderly interconnection of the granules. Occasional more oblong dark-staining elements appear. However, where the karyolymph is less solidly coagulated there is a strong suggestion of filaments connecting the granules with each other. This is to be interpreted as indicating that even back during the archesporial period of rest an actual though obscure system does exist anticipating the definite leptonema, not susceptible of direct demonstration in undisturbed form by the present technique, but betrayed by distorted strands imperfectly preserved when there is partial fixation of the karyolymph. There is little direct evidence that these granules are closely associated in simple strands, although it is clear that they may be connected in some fashion. The nucleus has meanwhile increased slightly, to about 14 μ in diameter.

The succeeding changes are concerned with the increasing definiteness of the spireme. Unfortunately the cells have now reached the point, for *Gasteria*, where synizetic contraction may occur, and the misleading effects of this phenomenon must be carefully discounted until, after synapsis, the cells cease to be subject to this disturbance. The general staining power of the granules decreases, so that some stand out sharply from the rest. Short bits of strand appear (Pl. XXVII, fig. 1). These become more marked and shortly in exceptionally favorable instances it is evident that the early leptotene structures which hold the stain are spiral (Pl. XXVII, fig. 2). However, they are so closely placed and so subject to lateral fusions and distortions with inaccurate fixation as to be very hard to detect, and the false impression received is that of an involved tangled thread

[2] Measurements were averaged from a number (at least 10) of characteristic cells in sections, usually of extruded microsporocytes, and usually from more than one fixation batch. They are given only as approximations to show relative sizes under the conditions of this study.

system. No chromosome vesicle or membrane was recognized as surrounding each spiral. The next stage involves a very rapid shortening of the spireme, the opening out of the spirals, and the transition to an interwoven thread system, which, as later leptonema, persists until toward the climax of synizetic sensitiveness. At mid-synizesis the tapetum cells are usually binucleate, and there is little or no degeneration evident in their cytoplasm. Occasional tapetal mitotic figures are evident. At the time the nucleus becomes subject to synizesis it is about 14.9 μ in diameter, and from this it grows rapidly to 17.7 μ or more before synapsis becomes well advanced.

This very important feature is hard to observe in *Gasteria*, but nevertheless the writer feels confident that it occurs by the passing of the leptotene strands into parallel relation in pairs. It is not difficult to find examples of short parallelisms in leptonema, but these alone are unconvincing. Favorable cases of amphinema, while uncommon, show a synapsis partially completed, the paired strands being evident on one side of the nucleus, leading to an unsynapsed part of the spireme on the other side, where the elements in the double strand diverge to become lost among the tangled simple threads (Pl. XXVII, fig. 3). Obviously synapsis is by lateral approximation of threads. It occurs while the cells are still strongly subject to synizetic contraction, and while the spireme elements are shortening very rapidly. As synapsis approaches completion, the cells lose their sensitiveness, and synizesis becomes progressively less of a disturbing factor. The synapsed spireme strands traverse the nucleus freely, and the nucleolus is still well preserved.

Up to this time there has been little evidence of definite organization within the spireme. The thread, though granular in appearance and often minutely nodulose in contour, has not shown clear-cut chromomeres. With the organization of the double spireme these elements become much more recognizable (Pl. XXVII, fig. 4). The associated threads generally show a tolerable correspondence in position, number, and size of the chromomeres over the comparatively short pieces which are in suitable position for simultaneous observation. Where the pachytene spireme strands are intimately conjoined the chromomeres may be exceedingly close together, and hard to resolve optically (Pl. XXVII, fig. 5). Free ends are definitely recognizable at this stage, though still somewhat difficult to locate because of the complexity of the system, which in its much greater involvement during earlier stages effectively prevented the forward extension of this observation (Pl. XXVII, fig. 4). There is now begun a continued rapid shortening of the spireme elements, with a tendency for long strands to lie against the nuclear wall, where they assume roughly parallel courses and seldom cross each other in close contact. Where exigencies of space might force too close a convergence one of the strands generally turns down to traverse the karyolymph. The spireme is definitely not limited to a peripheral position at this time, though much of it is peripheral, and this

actually corresponds to what has been described as the "hollow-spireme" stage. The nuclei of fixed extruded cells are about 19.6 μ in diameter. As the stage is very abundant it probably is a prolonged one.

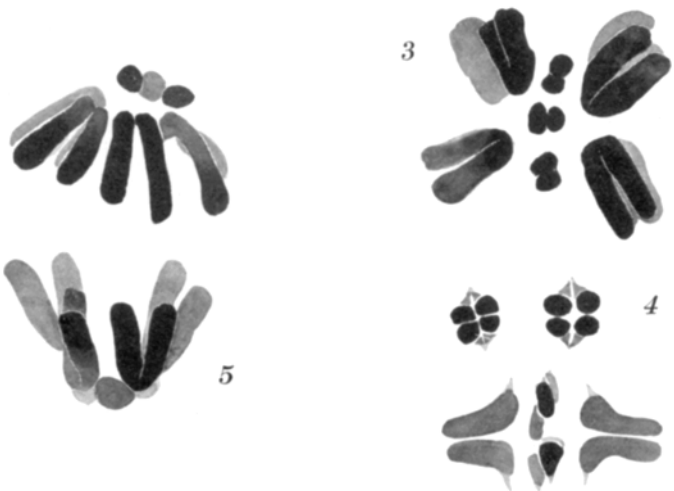
There follows a short and rather confusing period during which the synapsed threads separate somewhat widely. This diplonema is often accompanied by an apparent tangling about the nucleolus, and frequently paired loops appear to radiate from the tangled mass (Pl. XXVII, figs. 7, 9). Sometimes the process is not simultaneous throughout a cell, and the transition is seen (fig. 7). Chromomeres, at first distinct, become less numerous (fig. 8) and ultimately less distinct. The parallelisms of the peripheral pachynema were transitory and nothing comparable is now to

TEXT FIGS. 1, 2. FIG. 1, Mid-pachynema, threads not well enough preserved to show chromomeres and partially fused, but showing doubleness by the paired free ends. FIG. 2, Tetrads from one cell, representing three of the large chromosomes. × 2060.

be seen. As the shortening proceeds there becomes evident a considerable thickening of the threads, which was not marked during the earlier contraction.

The passage into strepsinema is rapid, but marked by no peculiar features (Pl. XXVII, figs. 9, 10, 11). Soon it appears possible to count the number of sets of elements present, though actually this can rarely be done with accuracy, since there is much overlapping (text fig. 1). In the best-placed bits of strand it is also possible to recognize a lateral segregation of their substance, with in effect the establishment of chromosome tetrads (Pl. XXVII, figs. 10, 11). At first this is evidenced by occasional short splits and seeming openings in the threads. Probably the continuity of the chromosome membrane is not altered by these, if indeed the tetrad has yet been established as a definite structure (Pl. XXVII, fig. 10). Later, as the strepsitene elements become shortened, definite separation shows two longitudinal chromonemata (Pl. XXVII, fig. 11). There is usually some slight spiral twisting, and very generally vacuolization of the matrix. Physical isolation of the chromonemata into separate chromatids is not immediately effected, but a well-marked tetrad is, though briefly, present (Pl. XXVIII, figs. 1, 2, 3; later, text fig. 2). The extruded cells at this stage have nuclei of about 17.1 μ diameter. The shortening does not

proceed to the extent of the formation of peripherally placed chromosome pairs in typical diakinesis before the breakdown of the nuclear membrane. Rather, the chromosome elements lie in the cavity, often with some tendency to clump in a vague contraction until the quick drawing in and dissolution of the nuclear membrane frees the chromosomes. During the period of crowding contraction of the tetrads is completed, the chromonemata each being apparently thrown into a spiral in its contracting chromatid. They are thus isolated in separate bodies which are at first closely placed side by side, but which separate as metaphase is established. Obviously, even if



TEXT FIGS. 3–5. FIG. 3, Polar view of metaphase plate to show the separation of the sister chromatids. FIG. 4, Lateral view of metaphase plate, two homologs, displaced for clearness sake, showing the tetrad structure of the homologous pairs. FIG. 5, Side view of anaphase figure. × 1470.

the process is a coiling one, each thread coils freely and separate from its neighbors, so that no difficulty due to interlocked parallel spirals exists here, in contrast to several other accounts, where it seems necessary to postulate some method of disentangling the involved coils. At first crowded together, with the dissolution of the nuclear membrane the chromosomes later spread out in the plate position, three small pairs near the center and four large pairs radiating therefrom (text fig. 3).

II. Metaphase

The lesser metaphase chromosomes are small rounded oval structures, somewhat elongated in the spindle axis, and slightly compressed parallel to their axes (text figs. 3, 4). Often it is difficult to distinguish more of their true constitution at metaphase, but from polar view it is generally clear that the compression is more fundamental than appears from the side, and one sees a fairly marked cleft on each face (text fig. 3). The larger

metaphase chromosomes are elongate, generally about 4 diameters long. One is from the first inclined to be bowed from the plane of the plate, the rest lying more closely side by side (Pl. XXVIII, fig. 4). All when viewed from the pole are definitely divided into closely placed chromatids, so that when viewed from the end each pair of homologs is unequivocally tetrad in its appearance as well as its constitution (text fig. 4).

The inner or spindle ends retain closer association than the outer ends, but are essentially separate structurally, however closely placed they may appear. When spindle fibers become recognizable it is found that one thickened 'tractor fiber' serves for each 'diad' pair of chromatids, large or small (Pl. XXVIII, fig. 4). On all but the one bowed pair of large elements the attachment is closely terminal, but on that one it is atelomitic, behind the tip, and related to the bowing of the element. As is indicated by their contour, and in the light of what has become known of their internal structure, the smaller chromosomes may be confidently assumed to be structurally divided at this time like the larger ones.

During the extreme concentration of the chromosomes when first freed from the nuclear membrane and because of the density of their interior, nothing has been clearly determined as to their structure by direct observation. When the plate has been organized for separation of the homologs conditions are very much changed. While the chromosomes are still extremely dense, it is easily established by critical differentiation in destaining that a distinction exists between the substances of which they are composed. At first only betrayed by the occasional crenellate appearance of the apparent margin, each is differentiated with further destaining into a dense core or matrix and a peripheral system resembling transverse denser rods (Pl. XXVIII, fig. 4). An accurate interpretation of most of the examples would be difficult were it not possible to work back from subsequent stages, and also were it not for an occasional more intelligible instance. In these favorable cells it is seen, and may be confirmed, that what at first look like transverse bars about the core, or like larger or denser disks alternating with smaller or less dense ones, really are turns of a spiral rod which winds about the chromatid or univalent metaphase chromosome at its periphery (Pl. XXVIII, fig. 4 p.p.).

Meanwhile, the spindle has been organized. Because of the character of fixation employed, which coagulated even the dilute karyolymph, the cytoplasm is so densely and evenly coagulated as to show comparatively little differentiation between the peripheral and spindle areas. However, the exterior region is definitely and minutely spongy in aspect, with no directional trend to its delicate reticulations, while the central or spindle region shows a striation roughly parallel to the axis of division even though it is equally smoothly fixed with the peripheral portion. This spindle ultimately assumes a biconical form. In conjunction with the chromosomes themselves a marked differentiation does, however, occur. At the apparent

traction point a denser structure appears, usually serving to attach the two chromosome halves, sometimes furcate, but single above and gently tapering off to merge with the more delicate spindle elements. In fixations with reduced osmic-acid content these attachment bodies terminate markedly heavy traction fibers, notably thicker than those of the bulk of the spindle, but where fixation is more complete and uniform and the spindle does not stain differentially with respect to its constituent parts, the distinction is harder to establish.

III. Anaphases

The first sign of activity on the plate may be marked by the spreading apart of the outer ends of one of the tetrad chromosomes (Taylor, 1929), with separation along the secondary split, not along the synaptic plane. Previously the halves of the tetrad have lain side by side, or bowed apart like stomatal guard cells, with the separation along the synaptic plane (Pl. XXVIII, fig. 4). The inner or spindle ends then move away from the plate position, retaining the juxtaposition of the diad chromosome tips while the homologs separate. The separation of the tips seems to pull the outer ends somewhat together before they finally separate, and the diads pass up toward the poles fairly parallel again. Usually one chromosome pair passes thus into anaphase before the others become active, but the remainder then move up together without marked divergence of their outer ends. It could not be absolutely determined which chromosome behaved differently from the rest, but since only one of the larger chromosomes is morphologically distinct it is probable that this (in the vegetative division the satellite-bearing) chromosome is the one concerned. While there is close approximation of the spindle ends of the chromosomes it is certain that natural separation of the halves has been complete before metaphase and that apparent partial joining is illusory, or an artifact from poor fixation (text figs. 3, 5). The chromosomes group themselves at the pole with the smaller elements in the upper center and the distal ends of the larger ones subparallel to the spindle surface.

As the chromosomes pass into anaphase they lengthen somewhat, and become much more easily differentiated into their constituent matrix and chromonema (Pl. XXVIII, fig. 5). A definite morphological membrane about these two constituents is apparently not demonstrable in early anaphase in undamaged chromosomes, but becomes more probable later. The presence in each half diad chromosome of one continuous chromonematal rod is perfectly obvious, it making 5–6 turns, either with right- or left-handed twist. Occasionally (as illustrated) the twist reverses its direction in part of a chromosome (Pl. XXVIII, fig. 5 p.p.). This is in harmony with the belief that the chromonemata of metaphase and later chromosome stages are formed by the twisting of the post-strepsinema filaments when the tetrad of chromatid threads is contracted in the clumping and condensation accompanying the dissolution of the nuclear membrane.

There has been a shortening of the spiral from the 7–8 turns present at metaphase (even more in the bowed atelomitic chromosome). Suggestions of doubleness rarely appear (Pl. XXVIII, fig. 6). There are suggestive evidences that the proximal ends of the chromonemata are folded back for a short distance parallel to the main shaft, and this flexure may well occur at the future fiber-attachment-constriction point, the folded part representing the "head" of the chromosome (Pl. XXVIII, figs. 5, 6, 7). If, as seems probable, this flexure has a purely mechanical explanation it need not be expected in chromosomes with median attachment. An indefinite biconcave zone of denser cytoplasm may sometimes be seen between the telophase chromosome masses, but this rarely is very evident and very rarely indeed, though then clearly, was a specimen seen where a heavy pectic wall had been formed between the two daughter nuclei.

IV. Telophases and Interkinesis

From this point two descriptions must be given, representing either conditions leading through an extended period of rest, or by contrast through a brief and partial dissociation quickly followed by the homoeotypic mitosis. No fundamental difference separates them, and indeed intergrades occur, but the aspects of the extremes were notably dissimilar. The more deliberate process may be described first. The chromosomes at the pole draw close together, widen and shorten. Each chromosome group revolves its axis through 90° until it lies across that of the spindle. There is no radial correlation between the positions of the two sets, each taking up its position across the old spindle axis at 90° without reference to the radial position of the other. The chromonematal turns draw together, and temporarily become indistinct. Then the incipient nucleus begins to expand rapidly, with the nuclear membrane probably already present, while the chromosomes may remain laterally approximate. By reason of rapid elongation and lateral connections the chromonemata quickly lose their spirality, and assume an irregular angularity accentuated by the lessened difference in stain-holding power between the chromonemata and the interconnections. The chromosomal chromonemata seem to become the spireme portion of the nucleus directly, and the matrix portion with any included cytoplasm to enter into the karyolymph. The whole nucleus loses in stain holding power, and the chromatic continuity of the spireme becomes interrupted. Boundaries between adjacent chromosomes are visible for a long time, since the spireme from each chromosome is mostly toward the center of its area, the "chromosome tract." Retention of a distinct membrane between each chromosome vesicle could hardly be affirmed, but the evidence is clearly for the maintenance of the individuality of the spireme from each chromosome and the maintenance of the relative positions of each chromosome with respect to the others. Ultimately the nucleus may develop an aspect of coarse sponginess with chromaticity retained at a multitude of

apparent intersections of alveoles or threads. No single large nucleolus is developed, although one to several little round nucleolus-like bodies do appear, probably of essentially the same nature. More often the homoeotypic prophases begin before the extreme stage of dispersal with the loss of recognizable chromosomal tracts has occurred.

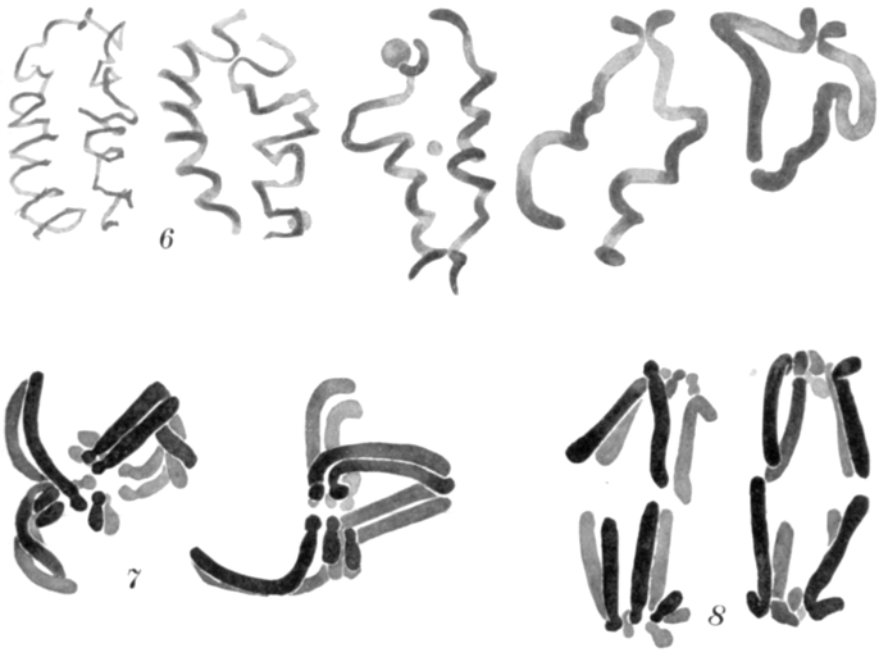
In the more accelerated procedure the chromosomes spread out rather widely upon reaching the pole, without making, or at most incompletely making, their 90° shift of position. A nuclear membrane then encloses the chromosome group and the adjacent cytoplasm, the latter immediately becoming more uniform in texture. The chromosomes enlarge considerably, the loops of the chromonemata increase in diameter, and become less strongly stainable. The definite boundary of the "chromosome vesicle" becomes vague, first at the distal end, and then throughout. The chromatin may be somewhat segregated in the chromonemata, giving a spotty appearance which, with the crossing of the chromonema, once induced the writer to report incorrectly that definite rows of chromomeres were present at this stage (Pl. XXVIII, figs. 8, 9, 10). The later telophasic stages were not available in this material, but the early homoeotypic prophases following it apparently may be normal. Instances have been seen, and will be reported upon by H.-C. Tuan, in which interkinetic dispersal and even telophases have been omitted, the cells proceeding directly into the second meiotic division from the first anaphase stages.

Second Maturation Division

V. Prophases

With the inception of activity the chromaticity of the nucleus again increases, and it becomes more granular in appearance under low power. The chromatin-bearing structures are mostly near the surface of the nucleus. Soon it is evident that there is a transverse arrangement of the chromatic structures, for a banding appears which, in the light of subsequent developments, may be designated as the reappearing of "chromatic tracts." A bit later very careful observation demonstrates that the stained elements tend to be longer across the axis of the tracts. Subsequently they appear to extend across them, and then it is found that they are connected together (Pl. XXIX, fig. 1). The structure thus disclosed becomes the homoeotypic chromosome by a simple condensing process. Each at first appears somewhat zig-zag, for the chromatin material is rather near the surface, but as the continuity becomes more obvious it seems to adjust itself to more regular loops (Pl. XXIX, fig. 2), and appears as a definite spiral which increases in symmetry to a marked degree (fig. 3). At first the close approximation of the several spiral elements produces an aspect of great complexity and confusion, but even then the limits of each spiral element can be traced for considerable distances (Pl. XXIX, fig. 3). As the elements shorten the

diameters of the spirals lessen somewhat and the elements become much thicker. Immediately it is possible to trace each element through its whole length one is able to recognize that the spirals always occur in pairs, closely approximated at one point (text fig. 6, Pl. XXX, figs. 1–4). The element beyond that point of juxtaposition is short or longer depending on the chromosome, for the point is readily traced through and found to develop early into a constriction which corresponds to the spindle fiber attachment constriction, and the part beyond the point is the head end of the chromosome, or the short arm according to the typical chromosome configuration



TEXT FIGS. 6–8. FIG. 6, Five diad or sister half-chromosome pairs, of successive ages, from cells in second meiotic prophases. The approximation at the point of future fiber-attachment shows, but no other structural differentiation. FIG. 7, Polar view and lateral view of two chromosome groups in second meiotic metaphase of the same microsporocyte. FIG. 8, Lateral views of two anaphase chromosome groups of the same microsporocyte. FIG. 6, × 2060; FIGS. 7, 8, × 1635.

in *Gasteria* (text fig. 6, Pl. XXX, fig. 4). The fact that the chromosomes come out of the homoeotypic prophases in the same general form as the spiral chromonemata exhibited in the heterotypic telophase, and in the same paired relation as the diads (heterotypic half chromosomes or univalent chromosomes) showed, and finally with the same polar interrelation, is one of the best possible confirmations of the continuity of the diad chromosomes in meiotic interkinesis.

To return to the early stages where continuity of the element becomes

clear: *i.e.*, as Pl. XXIX, fig. 2. It is evident that the element is of unequal thickness, and on careful examination in favorable places one notes that this is because two threads are involved, more or less parallel, and in part joined (Pl. XXIX, fig. 5*a*). In the early stages they are never regularly parallel. Later, in the continuous spiral stages the threads become too closely conjoined to permit of recognition. As the spirality is lost in late prophases the duality of each element becomes evident again in the often-compressed chromosomal structure with marginal, or at least double peripheral chromonemata (Pl. XXIX, fig. 5*b*). While the diad chromosomes remain attached at the point of future spindle attachment throughout the prophases, they diverge greatly toward the distal ends, which divergence becomes more striking as the process advances toward membrane dissolution (text fig. 6, Pl. XXX, figs. 1–4). As the prophases are completed the chromosomes are tolerably regularly distributed against the membrane, all their heads oriented toward one pole (Pl. XXX, fig. 5).

VI. Metaphase

The membrane disappears without much contraction of the mass, and the diad chromosomes draw close together into apposition, while they shorten somewhat. A general readjustment of position occurs to bring the head ends together, but now in the plate position (text fig. 7). The longer chromosomes cannot lie in the plane of the plate from limitations of space, so they curve away, some toward one pole and some toward the other, but the three short pairs lie on the plate level. Structurally the chromosomes show a condensation and increased twisting of the chromonemata, but these are by far less distinct than earlier (Pl. XXIX, fig. 5*c*).

VII. Anaphases

As separation begins the sister half chromosomes first part from each other at the constriction point, and arch apart until the head ends are separated. Then the shafts gradually separate. Ultimately the chromosomes stand straightly opposed, all the constricted ends at the poles and the heads pointing (in general) toward the axis. The length and diameter of the anaphase chromosomes varied very much indeed, being longer at first in those which are passing more slowly through meiosis. In any case the chromosomes shorten greatly as anaphase progresses. At least when they are initially attenuate they practically reach the spindle poles by the time that the distal ends have separated, so that anaphase is not so much a further passing to the pole as a contraction in length, with the head ends already in the final position (text fig. 8, Pl. XXIX, fig. 6). Structurally the chromonemata appear to straighten considerably (fig. 5*d*). This stage is harder to elucidate than the heterotypic anaphases. It approximates in structural character to the simple vegetative anaphases. Frequently the chromosomal core is much vacuolate and generally the stain is very firmly held throughout.

VIII. Telophases

When the contraction is complete the chromosomes draw somewhat close to the spindle axis and become enveloped by the new membrane (Pl. XXIX, fig. 7). The new nucleus then enlarges considerably, particularly in length. The chromosomes lengthen greatly, but do not take on a markedly spiral form, lying nearly straight against the nuclear wall with the chromonemata side by side as parallel bands (Pl. XXIX, figs. 8–10). Dispersion of the chromosomes is not rapid, but takes the course of gradual separation of the chromonemata, with a gradual loss in stain retention power of the chromatic part, so that the strands become pale and diffusely granular (Pl. XXIX, fig. 10). The chromosomal outlines become vague; the aspect of a greater density between the chromonemata disappears so that they seem to be free against the nuclear membrane unconfined by a chromosomal vesicle. By the time cytokinesis is complete it is difficult to designate the limits of the individual chromosomes in each nucleus.

IX. Cytokinesis

After the completion of the nuclear membranes there is established a fibrillar texture in the cytoplasm, radiating from each nucleus and connecting each with its neighbors. This shows best where the osmic acid of the fixing fluid has not done its work well. A clearing of the cytoplasm occurs midway between the opposite nuclei, the planes intersecting near the center. Centripetal cleavage occurs rapidly along these planes, followed by a slight rounding and separation of the protoplasts, especially near the center of the group. Then a membrane grows in, continuous with the gelatinous envelope about the whole quartet. On the completion of this the changes become more deliberate in the establishment of the young pollen grain.

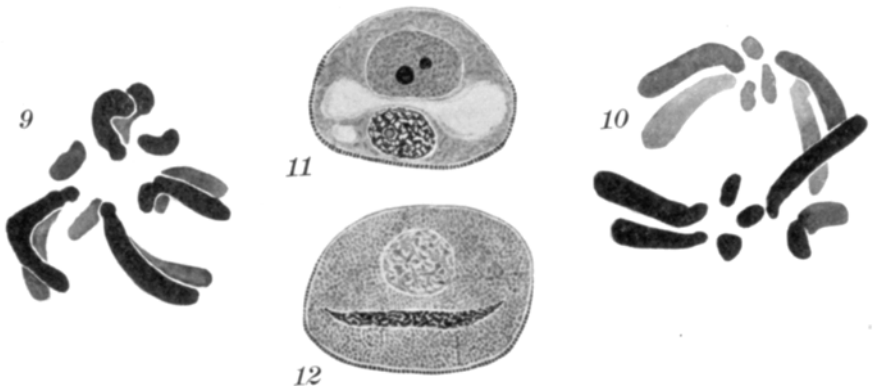
The peripheral cytoplasm of each cell becomes quite dense as a thin layer, and inside the gelatinous wall a thin, new membrane is formed. After this the gelatinous envelope progressively disappears. The chromatic elements of the nucleus complete their dispersal and the resting stage of the young one-nucleate pollen grain shows a spherical nucleus with irregularly granular contents and a large nucleolus. While some of the granules are moderately conspicuous they do not correspond to the number of chromosomes later formed.

XII. First Gametophytic Mitosis

Preservation of the contents of the *Gasteria* pollen grain by the haematoxylin smear method was very much superior to that secured by any other. Although the quality was not at all equal to that found in root tip cells, it gave a far more normal picture than usually presented by the sparse figures and descriptions of pollen grain mitoses that have been published, and so it seems worth while briefly to describe the process.

With beginning activity the diffuse granularity gives way to more coarse

granules, and these to irregular elongate strips of chromatic structure, the beginning of the spireme (Pl. XXX, fig. 6). Even with the somewhat imperfect fixation imposed by the heavy chitinous wall acting as a hindrance to penetration of the fixing fluid, the beginning spireme shows the duplex structure it inherits from the second meiotic anaphase. The continuity of the spireme strands becomes more evident and their contours more even, although they are much interconnected by anastomoses (Pl. XXX, figs. 7, 8). At first they are more or less twisted in form, or even spiral (Pl. XXX,



TEXT FIGS. 9–12. FIG. 9, Polar view of metaphase in first pollen grain mitosis. FIG. 10, Oblique lateral view of anaphase in first pollen grain mitosis. FIG. 11, Young pollen grain with beginning differentiation of nuclei. FIG. 12, Pollen grain with approximately mature nuclei.

figs. 9, 10, 11); later they become simplified to looped or bent rods, by which time the double character is obvious. The distinction between the large and small elements is finally very easy, and ultimately the nucleolus disappears (Pl. XXX, fig. 12). The small chromosomes generally lie together in the nucleus. With the breakdown of the nuclear membrane the chromosomes quickly form a very briefly maintained metaphase plate and then pass through the anaphase separation without a marked spindle (text figs. 9, 10). No internal structure was demonstrated in the chromosomes, but they showed shapes characteristic of the genus and most nearly like those of the second meiotic anaphase. However, this is, except for the absence of the distal satellite, practically the same as for the sporophytic vegetative chromosomes. There appeared nothing of note in the sparse telophasic cells seen. At first the two daughter nuclei are similar in appearance. Later they become separated by a vacuole, and the generative nucleus becomes granular, with sharp chromatic contrast, the tube nucleus more evenly and densely granular (text fig. 11). Finally, the generative nucleus elongates and the tube nucleus becomes less dense, while the cytoplasm fills up with stored food, displacing the vacuole and preparing the cell for transfer to the stigma and subsequent development there (text fig. 12).

Discussion

It is well to outline briefly the nature of the material which was used in this study, and the treatment accorded it. Greenhouse grown plants of several species of *Gasteria* were utilized, and no distinction was found in so far as this study was concerned. Where adequate the smear method developed by the writer was employed, with modifications suggested by Kaufmann (1927) and by Tuan (1930) after they became available. Because of the complexity of the spireme during mid-prophases and the coherence of the archesporium during early prophases the method was then unsuitable. Likewise at later stages, it sometimes failed when superposition of structures confused the images. The great virtue of the smear methods is that they bring the pollen mother cells into immediate contact with the killing fluid, so that if it is well chosen it does its work, unaltered by passing through varying numbers of cloaking cell layers, with precision, without causing shrinkage, and without permitting degeneration changes to occur. Where the above-mentioned reasons enforce the use of a section method, it is necessary to approximate the conditions which give the smear method its virtues. This was accomplished as far as possible for the present study by placing the androecium in a small quantity of the fixing fluid in a small wax-bottomed dish and immediately dissecting it as minutely as possible with very fine and sharp scalpels, dividing each anther into as many fragments as humanly possible within the few moments permissible. All of the fragments of a given fixation were assembled by pipette in a vial and handled by sedimentation through the succeeding stages, which in general involved close stages of alcohol concentration at short intervals, followed by alcohol-xylol intergrades, careful washing with well-dried xylol and as brief infiltration in paraffin at as low temperature as would give complete support. This mass of minute fragments yielded many small clumps of pollen mother cells extruded before they were affected by the fixing fluid, many isolated cells smeared over the anther surfaces, or anthers sliced open to yield areas of cells brought immediately into contact with the fixing fluid by the passage of the knife. Only such cells as had thus been immediately exposed to the fixing fluid were considered to afford reliable evidence for this study. Comparisons of cells in the center of an extruded mass, or in the center of an anther, with those at the surface of the mass or on the cut end of the anther abundantly confirmed the wisdom of this choice in the more perfect coagulation of the karyolymph, more precise contours of the spireme and chromosomes, their more regular and appropriate distribution in the space available to them, and their more intelligible internal structural differentiation. The fixing fluids used were various, but particularly modifications of Flemming's chrom-osmic-acetic solution with maltose and urea, which proved by far the most satisfactory. It is under just the circumstances provided here, with direct contact between cells to be studied and the fluid, that mixtures based on osmic and chromic

acids do their best work. The writer submits that no studies of meiosis with the mother cells enveloped by anther or sporangium tissues are likely to afford as truthful pictures as would have been had if the cells had been directly exposed, and doubts if the picture afforded under the more unfavorable conditions is often even approximately correct in detail.

In a *Gasteria* anther fixed intact the diameter of the nuclei was 19.4 μ at the climax of synizesis, with the synizetic mass 9.9 μ in diameter, showing a considerable increase in the volume of the nucleus since archesporial rest —from 950 μ^3 to 3823 μ^3—while the synizetic spireme and nucleolar mass occupied but 508 μ^3 of space, showing that under the unfavorable fixation conditions producing synizesis the spireme actually contracts from the archesporial space occupied, while the nucleus has enlarged. Very roughly, the nucleus has increased more than four times in volume. The changes probably involve a considerable intake of water, with a reduced concentration and reduced coagulability of the karyolymph. The spireme in organizing during this period seems to be already shortening, and this natural tendency is probably enhanced by special sensitiveness. Without the support of a simultaneously coagulating matrix at the time of killing the contractile spireme is left to condense into a tight knot, anchored at the point on the nuclear membrane where the coagulation began with the entrance of the fixing fluid, or about the nucleolus, or both. Throughout meiosis the slowly penetrating killing fluids and those acting by dehydration allow the chromatin-bearing elements to contract, and in early prophases more than later, when the karyolymph is again more easily coagulated. Increase of the proportion of osmic acid is a help in fixing these early stages, but this substance is comparatively ineffective if it has to pass through a succession of cells to reach the ones desired, and if disproportionately increased interferes with staining.

It should be emphasized that the permanent nuclear elements of the *Gasteria* cell are a number of threads which are recognizable as spireme strands or as chromonemata during cell division, but which, from limitations of technique, are not so surely recognizable during resting periods. It is not believed that these threads become discontinuous or really lose their identity or necessarily join together terminally during rest. Chromomeres may appear on these threads, and like the threads themselves may assume or lose the power of holding dyes. Irrespective of stainability, it is the chromonemata rather than the matrix or the membrane of the chromosome which are the parts definitely retained for transmission in division. Morphological differentiation of the threads is established, and chromomeres may be one feature of it, but they are so liable to artifactual irregularities that they must not be unduly stressed. It is to be noted further that the chromonemata or spireme threads are always double, or paired, structures, and that each division is prepared for before the last previous division is completed by the division of the chromosomes into two contained chromo-

nemata which will organize as parallel spireme elements for the split chromosomes of the next division. It is inferred in this account that the split which separates the elements to be isolated in the second meiotic division does not occur until late in the first meiotic prophases, but it may occur in the last archesporial anaphases and be optically unresolvable until recognized much later. The split in the chromonemata which will be isolated in the first post-meiotic division is evident in the earliest second meiotic prophases, and some evidence is offered for at least the beginning of the split in the first meiotic anaphase.

The significance of the spiral chromonemata may be involved, but one simple explanation appeals to the writer as probably adequate to explain their occurrence. Beyond certain limits longitudinal contraction of the spireme threads is somehow disadvantageous. Perhaps the actual physical seriation of its parts could not then be maintained. But it is mechanically more difficult to separate long and tangled chromosomes than short ones. So the spireme elements coil up as meiotic metaphase approaches, retaining the advantages of a long chromonema and those of a short chromosome. If the chromonema divides within the chromosome, at some stage before the split must become effective the daughter chromonemata are straightened until they are able to separate. The degree to which similar appearances may be expected in other plants may in part be predicted. Large-chromosome species will show them when the technique is adequate. Small-chromosome species do not offer as suitable material, and plants with many chromosomes do not have the same need for this mechanical adaptation. However, comparable structures and a comparable history are probably widespread in plants, though variations from this account, as well as corrections to it with improving technique, may be expected.

Summary

With inception of meiosis the initial granularity of the archesporial nucleus passes into indefinite short strands which, becoming more numerous and regular, develop as a number of spirals transversing the nucleus. The strands constituting these spirals, straightening, lose their relative positions and interweave through the nucleus. They then swing together in pairs, effecting synapsis, until the whole thread system is a double one. Chromomeres have appeared on the thread, and are generally found in similar pairs, but gradually again become indistinct. The paired threads, continuing to shorten, separate gradually from each other. It becomes recognizable that the number of pairs corresponds to the haploid chromosome number. Each element splits, forming a tetrad of four chromatids. A new shortening progresses very rapidly, and it appears that each thread is thrown into coils rather than shortened and a membrane develops about the spiral. The nuclear membrane disappears, and the metaphase plate is organized with three small homologous chromosome pairs of terminal spindle fiber

attachment, three large pairs with terminal attachment, and one large atelomitic pair. Each pair is obviously tetrad, and each chromatid contains a spiral thread or chromonema. Fourteen elements pass to each pole of the spindle, a diad of two sister chromatids from each tetrad side by side but clearly separate. A nuclear membrane forms about each group, and the new nucleus enlarges, the spiral coils of the chromonemata open out and gradually and irregularly lose their chromaticity, while the chromosome membranes become vague.

The second meiotic division opens with reassumption of chromaticity by the threads, which are found in spirals lying side by side, each spiral of two closely associated threads. These spirals shorten, and it becomes apparent that they are paired, each diad pair closely associated at the future fiber-attachment point. The chromosomes straighten out from their spiral form, but twist as they shorten so that the two associated threads—chromonemata—are spirally coiled within each chromosome. After breakdown of the nuclear membrane metaphase and anaphase stages are passed without special feature, except that the chromonemata are gradually uncoiled and come to lie fairly simply in the chromosomes. Spindle-fiber attachment constrictions are present during this division. After organization of the four quartet nuclei the chromosome membranes become vague and the chromonemata appear as parallel threads, or later as rows of granules, near the membrane. Ultimately they disappear and the nucleoli become prominent.

The nuclei of the young pollen grains show double threads as soon as mitotic activity is begun, and these shorten to form the haploid number of split chromosomes, which pass through mitosis in the usual way. Spindle-fiber attachment constrictions are evident, but the satellite seen during the diploid somatic mitoses was not noted. Chromonemata were not seen in the chromosomes either, but these features would be difficult to preserve in fixing through the thick wall of the pollen grain. The nuclei gradually lose their nucleoli, and the generative nucleus elongates, bringing the grain to the condition found at the time of effective pollination.

Department of Botany,
University of Michigan

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DESCRIPTION OF PLATES

PLATE XXVII

FIG. 1. Archesporial nucleus at inception of meiotic activity. × 2060.

FIG. 2. Parallel spiral leptotene threads in very early meiosis. × 2060.

FIG. 3. Synapsis from tangled leptonema (below) to pachynema (above). × 2060.

FIG. 4. Pachynema, approaching the hollow spireme condition, with chromomeres evident. × 1635.

FIG. 5. Pachynema, mostly at closest association of the threads. Portions of spireme, × 2060.

FIG. 6. Pachynema, artifactual representation of chromomeres as large, partly fused droplets on portion of spireme. × 2060.

FIG. 7. Pachynema, on the left, passing into diplonema on the right. × 1635.

FIG. 8. Pachynema, late, passing into diplonema. Portions of spireme, × 2060.

FIG. 9. Strepsinema, early, general view. × 1635.

FIG. 10. Strepsinema, late stage, general view. One element (lower right) beginning to show longitudinal segregation to form a tetrad. × 1635.

FIG. 11. Strepsinema, very late stage, with tetrad nature of some elements clearly recognizable. × 1635.

PLATE XXVIII

FIGS. 1, 2. Strepsinema, late, showing well marked tetrads with constituents well defined. × 2060.

FIG. 3. Strepsinema, late, showing tetrads. At *a* with the chromomeres ill preserved; at *b* in optical section, tetrad of small chromosome pair. × 2060.

FIG. 4. Metaphase (*a*, *b*) or incipient anaphase (*e*) chromosomes of the first meiotic division, polar (*f–h*) and side (*a*, *b*, *e*) views showing differentiation into matrix and chromonemata of various degrees of distinctness. × 2060.

FIG. 5. Anaphase chromosomes showing more obvious chromonemata with differentiation of future fiber-attachment point for second meiotic division, especially clear in the smaller chromosomes (lower right). × 2060.

FIG. 6. Anaphase chromosomes, later stage than figure 5. Note suggestion in lower right-hand specimen of a split in the chromonema. × 2060.

FIG. 7. Telophase chromosomes, early stage, showing increasing diameter of the spiral coils and lessened stainability. × 2060.

FIGS. 8–10. Telophase chromosomes, later stages, showing interrupted chromaticity. Figure 10 *in situ*, others selected elements. × 2060.

PLATE XXIX

FIG. 1. Interkinetic nucleus at inception of second maturation division, the chromatic tracts evident, with some suggestion of continuity. × 2060.

FIG. 2. Prophase of second maturation division, later stage showing spireme elements forming indefinite coils. × 2060.

FIG. 3. Prophase, later stage, with elements clearly spirally coiled. × 2060.

FIG. 4. Prophase, advanced stage, showing the diad split chromosome pairs in polarized relation. Note duplication in the two halves of the figure of the lower right-hand chromosome pair and half of the uppermost one, for orientation purposes. With these duplications the entire complement is illustrated. × 1635.

FIG. 5. Prophase spireme segments, and later chromosomes, to show duplex structure of the former and chromonemata in the latter. *a*, early prophase. *b*, *b*, breakdown of nuclear membrane. *c*, metaphase. *d*, *d*, rather early anaphases. × 2060.

FIG. 6. Anaphase chromosome group, lateral view to show form. × 1635.

FIG. 7. Anaphase chromosome group, very late. Chromonemata, well straightened, are in evidence, the matrix is vacuolated, and a hyaline area is developing about the group of chromosomes preparatory to the formation of the nuclear membrane. × 1635.

FIGS. 8–10. Telophases, progressive stages to show behavior of chromonemata. × 1635.

PLATE XXX

FIG. 1. Prophase, very advanced stage, showing chromosome pairs from the pole opposite that to which the head ends are directed. × 2000.

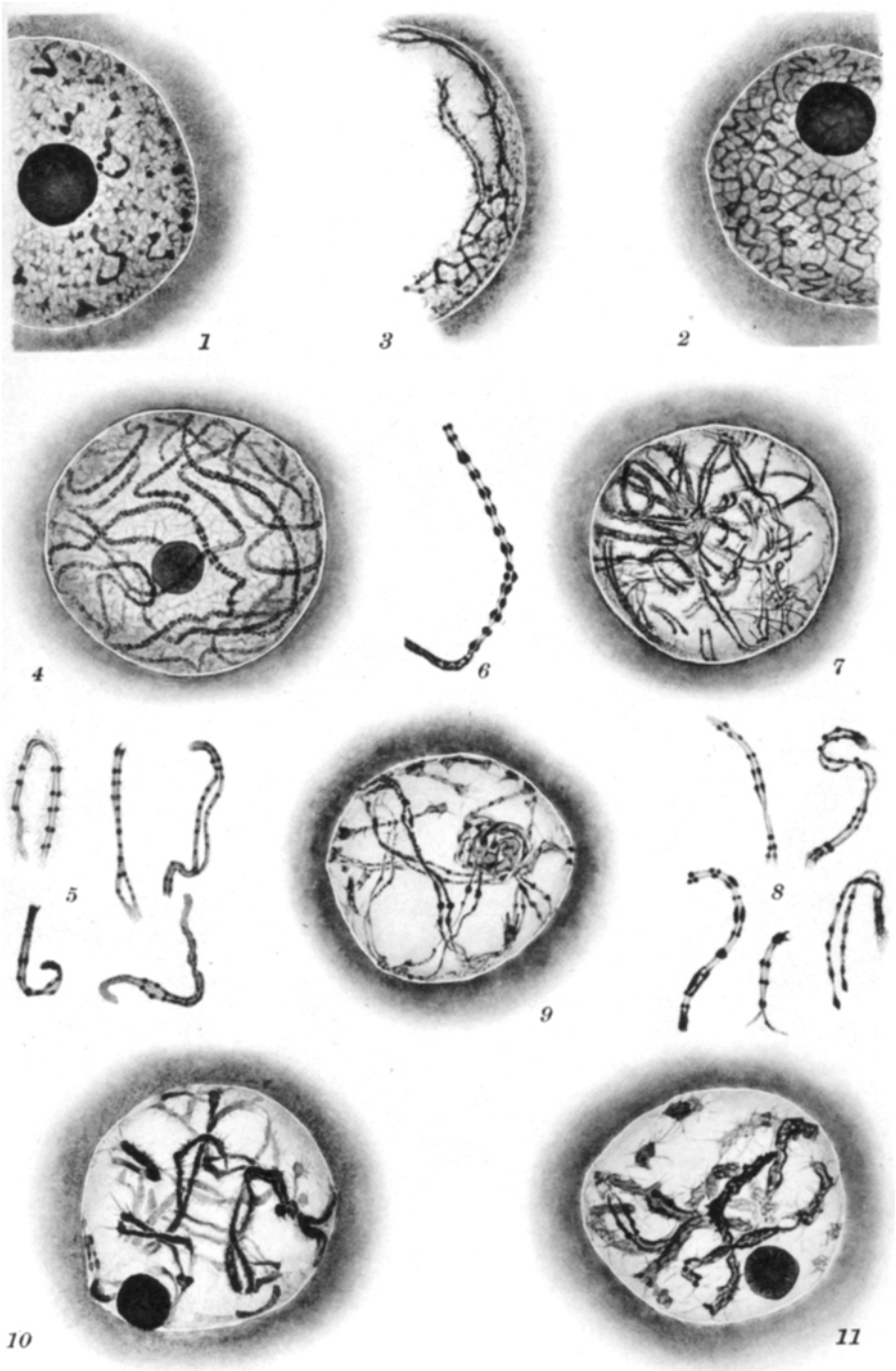
FIGS. 2, 3. Prophases, still more advanced, showing spindle-attachment ends of the split chromosomes. × 2000.

FIG. 4. Prophase, very late, with chromosomes almost straight, and the attachment-ends directed toward one pole. × 2000.

FIG. 5. Prophase, breakdown of the nuclear membrane. × 2000.

FIG. 6. Pollen grain, prophase of the first mitosis, early stage showing short chromatic bits of split spireme. × 2000.

FIGS. 7–12. Pollen grain, prophases of the first mitosis, successive stages showing gradual condensation and shortening of the split chromosomes. × 2000.



TAYLOR: GASTERIA


a
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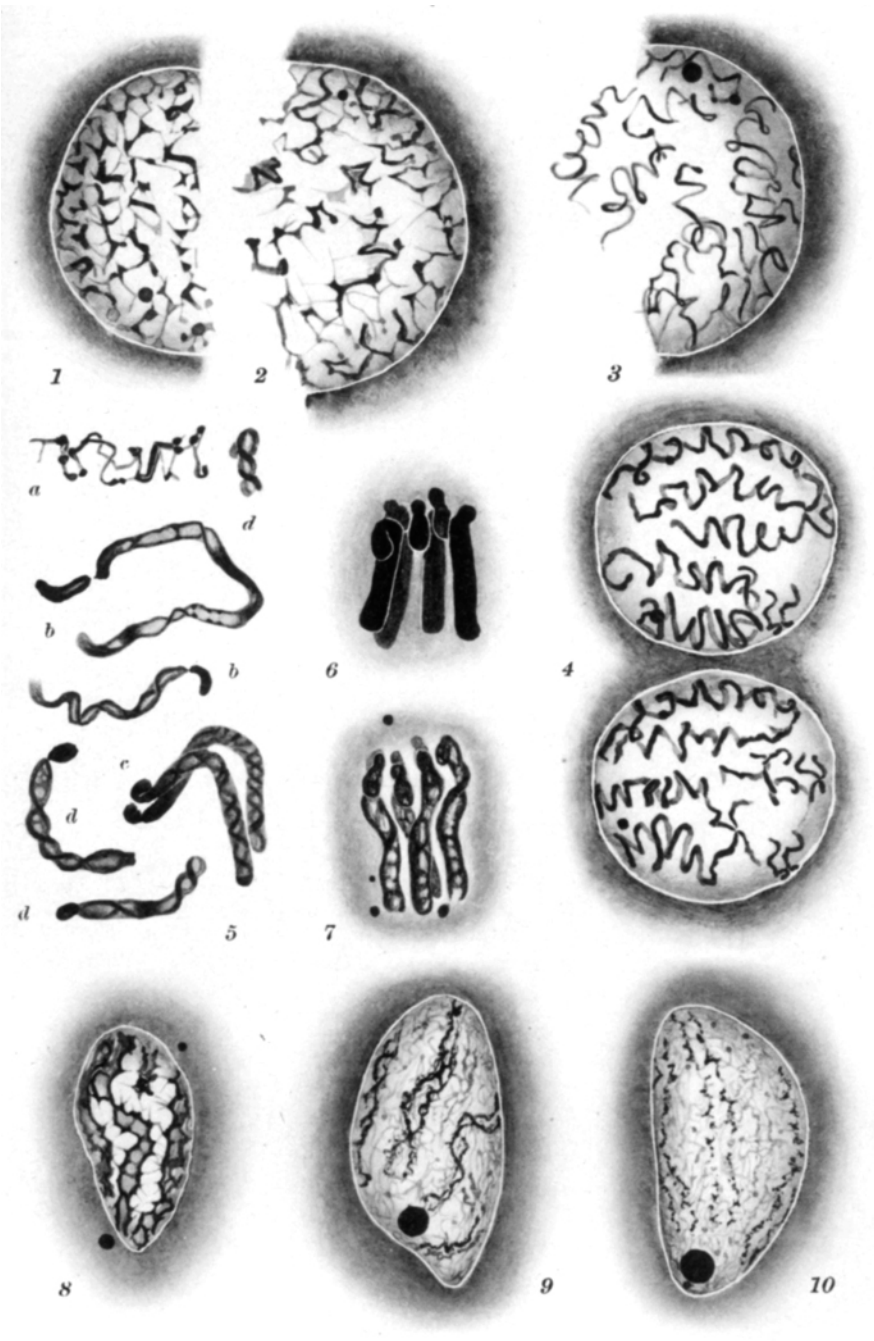

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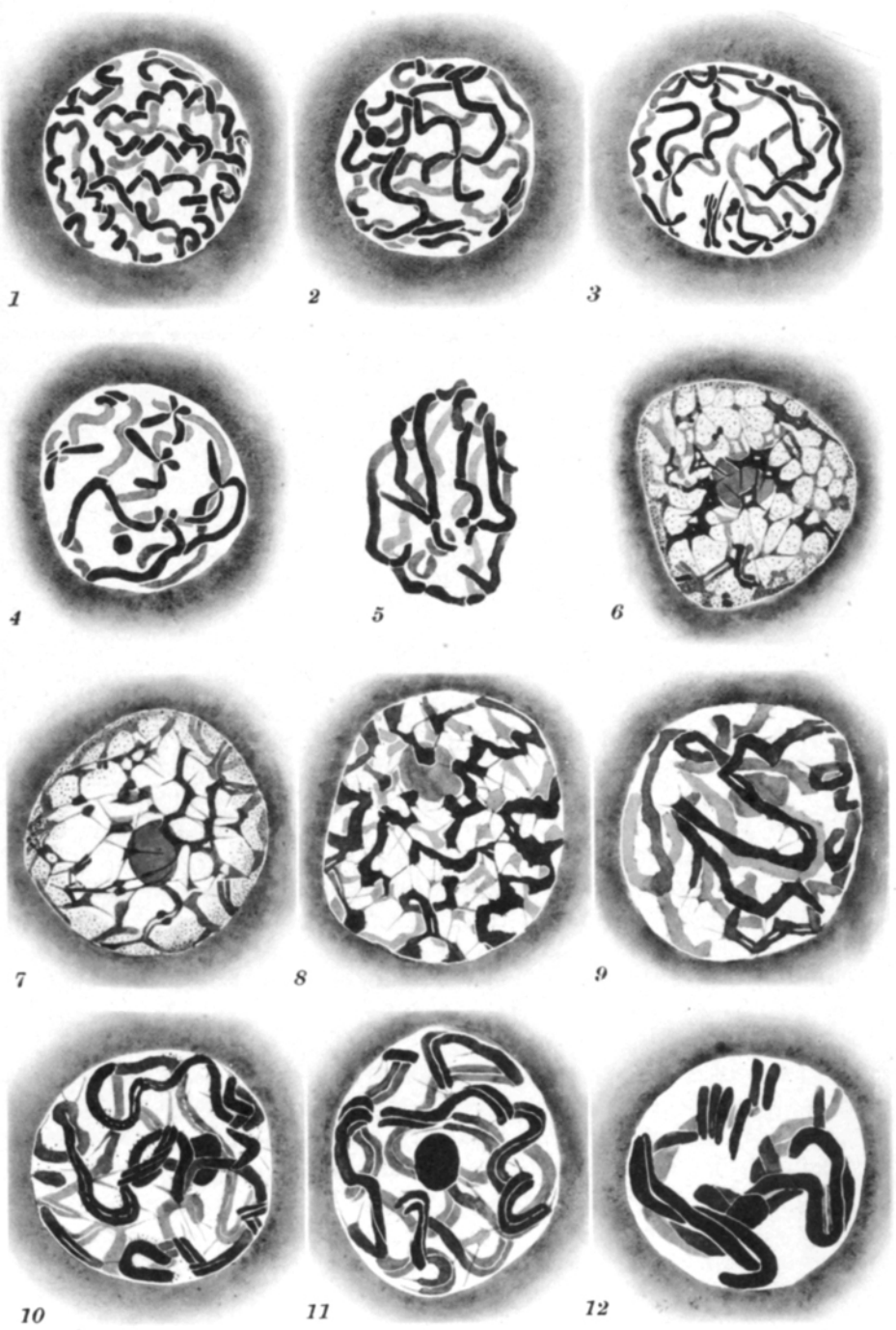

TAYLOR: GASTERIA