A CYTOLOGICAL STUDY OF COCOS NUCIFERA LINNÆUS

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SEVEN PLATES

For many years the reduction divisions in the mother cells have been studied and described in various forms with the object of determining the time, the nature, and the extent of the sequence of events during the process. The structure, the behavior, and the differentiation among chromosomes and the part they play in heredity have been also carefully considered. That the number of chromosomes is reduced to one-half previous to the union of the gametes became universally accepted, but the method by which reduction divisions or meiotic divisions are accomplished is still not so clear, because of the numerous diversified accounts and confusing views regarding the details of meiotic divisions, which need not be reviewed here. The diversity of opinion is partly due first, to the complexity of the process, coupled with certain unavoidable flaws in the technic essential to the study of the process; second, to the difficulty of interpreting the details of the series of stages under the condition in which the delicate material is studied; and, third, to the specific differences exhibited by the various organisms during the meiotic divisions. Any additional cytological study, therefore, is of considerable interest. Since the anthers of *Cocos nucifera* Linnæus are rather favorable material for such an investigation, this piece of work was undertaken with the hope of throwing more light upon the phenomenon of synezesis, or synapsis, and its relation to reduction division.

MATERIAL AND METHODS

The material used in this study was collected from two places in Luzon, Philippine Islands, where the tree is extensively cultivated. One portion of it was collected on April 7, 1926, from Mauban, Tayabas Province, while the other and the greater portion was collected on May 13, 1926, from San Pablo, Laguna Province. The two sets of material were fixed in the field from the inflorescences just beginning to emerge from the axils of the leaves. The samples were taken from the different parts of the inflorescence and fixed separately. Thus, those taken from the tips of the spikes were fixed separately from those taken from the middle part and separately from those collected from the base.

The fixing fluids used were 1 per cent chrom-acetic acid with ten to fifteen drops of 1 per cent osmic acid to 50 cubic centimeters of the solution, and Flehmming's strong chrom-osmic acetic acid solution. The young anthers were mostly cut into two parts and some were trimmed at both ends of the anther before they were placed in the fixing fluids. Both fixatives gave fairly satisfactory results.

The material was embedded in 50° to 52° C. paraffin, and cut from 5 to 10 μ thick with some 10 to 15 μ thick and stained with Haidenhain's iron-alum hæmatoxylin.

RESTING STAGE TO SYNEZESIS

The pollen mother cells following the last mitosis in the archesporium are more or less polygonal in outline and fit tightly together with no spaces between them. There is as yet no rounding of the cell wall. The cytoplasm is exceedingly dense and granular with a very few small vacuoles. The nucleus is comparatively large, somewhat spherical, and possesses a well-defined nuclear membrane. It is usually found lying very close to one side of the cell, especially during prophase and synezesic stages; but soon after the spireme begins to emerge and spread throughout the nuclear cavity, the nucleus seems to move toward the central part of the cell. There were comparatively few cases in which the nucleus was found close to one side of the cell during the open spireme stage. Even in such cases, the position of the nucleus compared with that in the earlier stages is farther from the wall.

The scarcity of the resting stage of the nucleus, during the early stage of the anthers of *Cocos nucifera*, seems to indicate that it lasts only for a short time, for it soon goes into prophase. Plate 1, fig. 1, represents a definite resting stage. It is characterized by the somewhat uniformly distributed chromatin reticulum throughout its cavity. At the intersections between the chromatin threads and the delicate fibers connecting them there are very minute nodelike structures or granules of different sizes. During this stage the chromosomes were completely resolved into a network of threads and their individuality is completely lost. There is no trace whatever of parallelism of the threads.

Heterotypic prophase.—The early prophase of the first meiotic division is differentiated from the resting stage by the gradual and progressive increase in size of the chromatin granules or nodelike structures in the reticulum, the thickening and contraction of the threads, and the decided enlargement of the nucleus. As the nuclear changes advance, the granules come closer together and take a deeper stain, while the chromatin threads contract and become thicker and shorter. The reticulum as a whole loses the even and uniform characteristic, as represented on Plate 1, fig. 2. The meshes become larger and different in size. Some of the threads which have been lying far apart are apparently brought near together by their gradual contraction. Plate 1, fig. 3, shows the condition of the reticulum before synezesis. The chromatin threads are irregularly thickened, the nodal structures are more prominent, and the meshes become smaller.

Synezesis.—The gradual withdrawal of the chromatin threads, or network, from the nuclear membrane marks the beginning of synezesis or synapsis as represented on Plate 1, fig. 4. During the progress of events leading to this stage there is a notable and irregular progressive increase in diameter of the threads, which seems to be brought about not only by contraction but also by fusion of material from the chromatin threads that happen to run parallel to one another. Furthermore, there is an indication of a flow of material from one thread to another, or from one part of a thread to another part, similar to what Cleland(5) observed in *Oenothera franciscana*. A greatly enlarged portion of the thread is represented on Plate 1, fig. 5. The contraction continues steadily until almost all the threads and meshes in the reticulum are drawn into a more or less compact mass that lies usually near the side of the large nucleolus and at one side of the nuclear cavity. At this stage the chromatin material appears as a lumpy mass with some globular projections, and a few small loops may be found on one or two sides of the synezesic knot. The pollen mother-cell nucleus remains in this condition for a long period, for the majority of the nuclei of the pollen mother cells of the younger anthers are usually observed in synezesis. A series of stages leading to synezesic contraction are represented on Plate 1, figs. 6 and 8, and on Plate 2, figs. 9 and 10. Very frequently fine strands extend across the nuclear cavity. Plate 1, fig. 7, shows a portion of a greatly magnified spireme taken from the nucleus represented in fig. 6. Figure 10 represents a definite stage of synezesis after a maximum contraction is attained. The nature of the spireme at this stage is very difficult to determine with accuracy. A careful examination of some thin tangential sections, however, seems to reveal that the chromatin nodes, or granules, were brought very close together by contraction. These granules are found arranged in most cases in single rows and in a very few instances in two rows running side by side. Since these few cases of the parallel arrangement of the granules are so rare in the material examined it is quite obvious that the spireme is univalent in nature. From this fact the condition observed in *Cocos nucifera* can by no means be compared either with Digby's(9) findings in *Osmunda* or with the writer's(20, 21) in *Elodea*, in which the parallel arrangement of the granules or the double nature of spireme is very evident. All the indications tend to agree with the observations of Cleland(5) on *Oenothera*, in which the scattered chromatin granules from a thin-thread reticulum are brought together into a thick spireme by a process that consists largely of the contraction of certain threads and the absorption of their contents into the body of other threads, a process which he believes involves occasional parallelism. Plate 2, fig. 11, represents a very small portion of the spireme in the same stage as fig. 10.

That the synezesis is brought about by the contraction of the chromatin reticulum and by the decided enlargement of the nucleus, as has been definitely observed in *Lactuca* by Gates and Rees(13) and in *Elodea* by the writer(20, 21), is also very evident in *Cocos nucifera*. When the size of the contracted reticulum is compared with that of the resting condition and the cavity of this nucleus with that of the synezesic stage a relatively great difference is noted. The synezesic knot is distinctly smaller than the original reticulum, while the cavity of the resting stage is comparatively smaller than that of the synezesic stage. This is essentially contrary to Lawsons's view point.

The nucleolus may, or may not, be entirely inclosed by the chromatin knot. Generally, however, it is found at one side of the synezesic knot, and sometimes is partly covered by a few projecting loops of the spireme. There is no material change observed in the nucleolus up to this stage except that it has increased in size, almost twice as much as during the resting stage.

Open spireme.—After the period of the greatest contraction, the chromatin mass begins to loosen and it becomes a continuous univalent short and thickened thread, known as the spireme, which extend slowly throughout the nuclear cavity. Free ends are rarely observed. During this gradual distribution of the spireme, beautiful loops corresponding to the gametic number of the chromosomes are formed. At this stage the spireme appears more uniform in diameter, although in some places it may have an irregular thickening. Plate 2, figs. 12, 13, and 15, illustrates this gradual unfolding of the spireme from the synezesic knot. Judging from the frequency of this stage in the several sections examined, it appears to be of long duration. There is no sign of splitting observable, and the spireme, unlike that of *Elodea*,(20, 21) does not show the double nature. The spireme is distinctly single and more or less smooth and uniform. Plate 2, fig. 14, is a detailed drawing of a greatly magnified portion of the spireme taken from a superficial section.

While the spreading of the spireme is taking place the nucleolus begins to separate from the spireme and its size is gradually reduced. This reduction of the size of the nucleolus is significant. It seems that the nucleolar content contributes in someway to the formation of the thick spireme. Cleland(5) shows a figure that indicates a clear connection between the nucleolus and one end of the spireme. It is also quite evident that the nucleolus at this stage takes the stain very lightly, and a few clear rounded spaces are developed.

Second contraction.—Shortly after the spireme is uniformly spread into wide loops radiating throughout the nuclear cavity (Plate 3, fig. 16), the nucleous passes into another interesting phase of meiosis known as second contraction. Early stages leading to this phase are represented on Plate 3, figs. 17, 18, and 19. The first indication noticed is the gradual shortening and approximation of the sides of each loop and the progressive thickening and condensation of the chromatin thread. Up to this stage the chromatin thread remains distinctly single and there is no sign at all of fission.

As the process of shortening and condensation proceeds, the loops become shorter and thicker and their two sides approach each other closer and closer, while the spireme as a whole undergoes a slow process of transverse segmentation. The segmentation becomes evident from the time the loops begin to rearrange themselves in the nuclear cavity, and it is apparently completed during the later stage of the second contraction. It is indicated by the formation of a constriction usually at the distal end of the loop (Plate 3, figs. 17 and 18). The loops are gradually detached while the massing of the filaments is going on. In a critical study of the chromatin filaments at this stage, particularly on the two sides of the loops, the writer failed to find any sign of longitudinal splitting or fission. This shows that each side of the loop represents a univalent chromosome, contrary to the interpretation of Gregoire (14, 15, 16) and his followers.

While the nucleus advances into second contraction, the greater part of the spireme filaments become gradually collected and entangled at the side of the nucleolus. During this period the visible identity of the individual parts of the spireme is somewhat lost, although in some cases isolated portions may remain independent and free. Plate 3, figs. 20 and 21, shows the character of the late stages of the second contraction in *Cocos nucifera*.

It is interesting to notice that during the later period of the second contraction there is a reduction in size of the nucleus. The pollen mother cells, which during the resting stage up to the later stage of synezesis were packed tightly together, are beginning to round off and separate from one another and large spaces among them are developed.

Diakinesis.—The massing of the chromatin material during the second contraction is of short duration only, for it soon loosens and proceeds into diakinesis. This is perhaps the reason why this stage was overlooked by some of the previous investigators. As the nucleus advances into this stage, the bivalent segments are gradually thrown toward the periphery of the nuclear cavity and they begin to show a somewhat clear outline. On Plate 3, fig. 21, the bivalent chromosomes in varying stages of evolution can be seen emerging from the knot of the second contraction. On one side of the nuclear cavity are three distinct loops coming out from the knot and moving toward the periphery, while on the other side one or two loops are in the process of organization. Some of the chromosomes are irregularly thickened and more or less spirally twisted along their entire lengths, others show distinctly a double character, still others are slender and have a somewhat uniform diameter. In the last the chromosomes are univalent but joined end to end. During this period of nuclear activity the chromosomes usually tend to assume a position close to the nuclear membrane. This peripheral arrangement is especially conspicuous after considerable thickening and condensation of the chromosomes have occurred. The chromosomes represented on Plate 4, fig. 22, show a definite stage after the second contraction. On the same plate fig. 23 illustrates a more-advanced stage in which most of the young heterotypic chromosomes are lying close to the peripheral part of the nucleus.

While the paired chromosomes grow thicker and shorter the daughter chromosomes, or the members of the pairs, begin to separate or untwist. This process may be followed in a series of stages represented on Plate 4, figs. 23, 24, 25, 26*a*, and 26*b*, and Plate 5, fig. 27. The usual result of this separation, or untwisting, is the evolution of the configuration of the chromosomes into various shapes. In some cases they become more or less like J's, O's, X's, and Y's, but in very many cases they are found having the characteristic shapes of U's and V's. A few fine and delicate strands are observed connecting separate chromosomes, and some run from the chromosomes to either the nuclear membrane or nucleolus. These strands are obscurely granular, and they may appear straight, curved, or crooked. Sometimes they connect each other and form a sort of net (Plate 4, fig. 26*b*). The chromosomes now appear more homogeneous in structure, and they take more uniformly a deeper stain. Their outline is somewhat undulated, there is no splitting, and a great diversity is shown in their length. Plate 5, fig. 28, represents three greatly magnified bivalent chromosomes with Y, U, and X shapes and in the same stage as Plate 4, fig. 26.

Plate 5, fig. 29, represents an interesting stage after the greater contraction and thickening have occurred. The chromosomes present a ragged appearance. They are mostly V-shaped, although some possess either U or ring forms. There is no material increase in the number of strands connecting one chromosome to another or the chromosomes to the nuclear membrane. Still later stages of diakinesis are shown in figs. 30 and 31. The paired chromosomes appear definitely individualized with a more-distinct outline. Some of them simulate dumb-bell-shaped bodies, and others, straight and bent rods. As in the earlier stages, they are of different lengths and each chromosome is composed of two short structures, so closely appressed to one another that its double nature is often difficult to detect.

The number of bivalent chromosomes at this stage of the nuclear development was carefully considered. Several counts from different sections and from different preparations were made. In general the bivalent number observed was sixteen, and in a very few instances fifteen. These few exceptional cases, however, can be accounted for as due to the frequent overlapping of the chromosomes.

During the late diakinesis stage and previous to the dissolution of the nuclear membrane, there is a notable decrease in size of the nucleolus and also of the nuclear cavity. The shape of the nucleolus, as well as of the nuclear cavity, is apparently modified from spherical to elongated or ovoid. The nucleolus, which stands out prominently during the earlier stages of the pollen mother cell because of its great affinity for stain, begins to show a marked decreasing affinity for the stain. Its nucleolar content is greatly reduced as shown by the fact that two or more clear round spaces or intra-nucleolar vacuoles are developed.

The nuclear membrane becomes more or less granular and irregular or wavy in outline. Its definite and smooth appearance is gradually lost, while the cytoplasm around it becomes denser and denser. That the nuclear membrane and the nucleolus disappear simultaneously at the time the fibers with granular appearance emerge from at least the three sides of nucleus is quite evident, as has been observed in *Elodea*.(20, 21) Plate 5, figs. 32 and 33, represents two characteristic stages, one closely following the other, of the late diakinesis. The multipolar spindle fibers are granular in structure and quite distinctly attached to the definite parts of the chromosomes. As the formation of the spindle fibers progresses, the nuclear cavity becomes smaller until it is completely filled by the fibers and by the dense cytoplasmic material. The chromosomes aggregate into a somewhat close irregular mass at the center of the pollen mother cell.

HETEROTYPIC MITOSIS

As the multipolar spindle fibers grow in number they are gradually rearranged into bipolars as indicated on Plate 5, fig. 34. The spindle fibers are seen stretching and have lost their granular character, while the chromosomes, which by this time have undergone a complete process of condensation, are moving toward the equatorial plane and they appear as bent rods, pestlelike, or somewhat globular. The chromosomes seem not to reach the equatorial region at the same time, but as soon as they reach this region, they become arranged more or less evenly in one plane and in a very symmetrical manner.

A polar view of the heterotypic chromosomes is shown in fig. 35. The sixteen bivalent chromosomes are very evident, and they display the same variation in shape. One of the sixteen heterotypic chromosomes is strikingly larger than the others, and the longitudinal fission between the two daughter chromosomes is very distinct, while three other chromosomes are smaller in size. They appear somewhat pestlelike and do not show longitudinal fission. This is one of the most favorable stages for counting the chromosomes for they are not crowded and seldom overlap each other. The several counts made at this stage revealed the same prevailing number, sixteen, that was obtained previously from the younger stages.

At the equatorial plate the heterotypic chromosomes may be arranged radially or obliquely. Each chromosome has a small bundle of spindle fibers attached to the inner end of each daughter segment, and the outer end extends perpendicularly from the spindle to the cytoplasm. In some cases, however, the chromosomes lying near the periphery or near the central part may be found arranged tangentially to the spindle. In this case the point of attachment of the spindle fibers to the daughter chromosomes may be at either end or at, or near, the middle region.

A typical side view of the metaphase stage of the heterotypic mitosis is represented on Plate 6, fig. 36. The general appearance is as if the contraction of the spindle fibers gradually pulled the two longitudinal halves apart. At the middle part, between the two separating daughter segments of each bivalent chromosome, there is a thickening which consists of the two ends that have not yet separated. Apparently, judging from this figure, all the chromosomes divide at the same time.

Soon after the daughter chromosomes have separated they immediately diverge at their equatorial ends, and take on the V-shaped or, sometimes, the J-shaped forms, with apex toward the pole. As they move toward the poles, they pass through a slight process of condensation and differentiation until they reach the poles. In some cases a sign of splitting preparatory for the homœotypic mitosis is observed. Plate 6, fig. 37, represents an anaphase stage. The V- and J-shaped chromosomes are midway to the poles. A late anaphase is indicated in fig. 38. It illustrates the stage just after the daughter chromosomes have reached the poles. At this stage the chromosomes still retain their V- or J-shapes, although in some cases, their two ends are somewhat separated and appear as bent rods. Figure

39 shows a polar view of the sixteen univalent chromosomes at about the same stage as that of fig. 38. The fission of the chromosomes in preparation for the succeeding homœotypic mitosis becomes evident as they are congregated at the poles of the spindles. As the daughter chromosomes gather and mass together at the poles, they become surrounded by dense cytoplasm. Then a clear space, or vacuolelike region, developes between the chromosomes, the boundary of which becomes the membrane of the daughter nucleus. This stage is represented on Plate 6, fig. 40, a telophase stage of the heterotypic mitosis. While this clear space and the nuclear membrane are in the process of organization the chromosomes expand and become longer and thinner. They radiate outward across the flattened region of the reconstructing daughter nucleus. Their free ends begin to curve, and because of this curving the end of one arm of a chromosome is brought into contact with an arm of another chromosome and those ends apparently fuse together. This fussion of the end of one chromosome to another seems to form a spireme of short duration only, for it is soon transversally segmented. This is shown by the fact that in the succeeding stages, although the shape and the position of the chromosome are apparently retained, the writer failed to find a distinct continuous spireme among the sections examined. Plate 6, fig. 41, represents a stage closely following the one shown in fig. 40. The nuclear membrane is definitely formed and the daughter J- or V-shaped chromosomes are seen connected with one another by delicate strands. Still more advanced stages of the reconstructing daughter nuclei are indicated on Plate 7, figs. 42 and 43. The daughter chromosomes with somewhat ragged appearance and partially condensed are more or less evenly distributed in the nuclear cavity. The nucleus continues to grow in size and becomes rounded. The nucleolus is relatively small and frequently is hidden between the chromosomes and does not appear clearly in the daughter nucleus.

HOMŒOTYPIC MITOSIS

The succession of events during the division of the daughter nuclei is extremely rapid. The period covered from the time the nuclear membrane is formed in the late telophase, up to the reorganization of the daughter chromosomes preparatory for the second division is of short duration. The daughter nuclei appear not to pass into anything resembling a typical resting condition.

Shortly after the nucleus has sufficiently increased in size and assumed the spherical form and the chromosomes have attained a more distinct outline, the nuclear membrane breaks down and the multipolar spindle fibers appear at different sides of the nuclear cavity. The cytoplasmic materials diffuse into the nuclear cavity, while the chromosomes collect closely together, as in the previous division. The spindle fibers radiate into this mass, and each chromosome is attached at, or close to, its end by a small bundle of fibers. As in the heterotypic division, the multipolar spindle fibers become bipolars while the chromosomes become arranged in the equatorial plate. Plate 7, fig. 44, shows a polar view of the two sets of chromosomes more or less evenly distributed. There are sixteen monovalent chromosomes in each set. Like the heterotypic chromosomes they display various shapes; some of them are somewhat globular while others resemble short bent rods, but most of them are pestlelike or dumb-bell-shaped.

The two spindles may lie parallel or at right angles to each other, or in some cases in a V-shaped position. Plate 7, fig. 45, represents a side view of two spindles at the metaphase stage lying almost parallel to each other. Figure 46, on the same plate, is about the same stage as fig. 45 and shows a side view of one spindle and more or less of an oblique polar view of the other spindle. This indicates that the two spindles lie somewhat at right angles to one another. The majority of the chromosomes seem to be attached at the end or very close to it. They exhibit the same variations in shape as did the daughter chromosomes, which were observed in the equatorial plate, appearing during their separation in the heterotypic mitosis. As the granddaughter chromosomes move away from one another and proceed toward the spindle poles they become V-shaped as in the previous division, but as they approach the poles they become considerably straightened out. During this stage no fission was observed. At the poles the chromosomes draw together in a mass and become surrounded by a denser cytoplasm, which is followed by a gradual expansion and the appearance of a clear space between them. Ultimately, the nuclear-limiting membrane makes its appearance, while the chromosomes advance into a process of alveolization. Plate 7, fig. 47, represents a typical telophase stage just before the formation of the nuclear membrane and nucleolus, while fig. 48 shows one of the granddaughter nuclei after the nuclear membrane and the nucleolus made their appearance.

DISCUSSION

The series of changes preceding the development of the heterotypic spireme up to the formation of the bivalent chromosomes have been the important subject for research and debates for the last twenty-five years. Much work has been done and published on the cytology of animals and plants. Various accounts and interpretations of reduction division were offered and as a result two important theories were advanced; namely, parasynapsis and telosynapsis. Both of these theories are very conspicuous in recent literature. The parasynapsis interpretation may be said to have been initiated by the excellent work of Hans Von Winiwarter(25) and strongly supported by Gregoire,(14, 15, 16) A. and K. E. Schreiner,(23, 24) Allen,(1) Berghs,(2, 3) and others. According to this scheme the spireme consists of a double-thread system which arises by the union of homologous parental elements during the heterotypic prophase. Thus, the resulting spireme is bivalent in nature, and each of its segments is composed of two somatic chromosomes arranged side by side. The telosynaptic interpretation was advanced by Farmer and Moore,(10) and supported by Fraser,(11) Mottier,(19) Digby,(9) and others. The double-thread system in the heterotypic prophase, according to this scheme, arises by the pairing of the threads derived from the halves of the somatic chromosomes which have split by process of alveolization. Therefore, the resulting spireme from this pairing is univalent in nature, and any segment of it consists of a single somatic chromosome and the union of the paternal and maternal chromosomes takes place at the second contraction. A slight variation of this scheme, however, has been observed in *Oenothera*, by Gates,(12) Davis,(6, 7, 8) and Cleland;(5) in *Fucus* and *Cutleria*, by Yamanouchi;(26, 27) and in *Lactuca*, by Gates and Rees.(13) The spireme in the heterotypic prophase is observed as a single unsplit filament, which arises by an irregular process of condensation, and the split preparatory for the second contraction is first observed only in the heterotypic anaphase.

The foregoing description of the reduction division of *Cocos nucifera* presents strong evidence for the telosynaptic interpretation. During the early heterotypic prophase the chromatin threads are arranged in a very irregular manner and they are mostly found unpaired. Although in some cases it is possible to find threads that run closely parallel to one another for some distance, the degree of parallelism is too insignificant to amount to anything in support of the parasynaptic interpretation. This occasional parallelism of the threads may be considered as mere chance, occurring in certain conditions as presented by *Cocos nucifera*. All my observations seem to corroborate the accounts of Gates,(12) Davis,(6, 7, 8) Gates and Rees,(13) and Cleland,(5) in which the spireme in the heterotypic prophase is said to be formed by an irregular process of condensation rather than by the union of two distinct threads.

During the later period of the synezesic contraction the chromatic knot appears very complex and the nature of the spireme is rather difficult to determine. This is the most critical period of the life-cycle of the heterotypic mitosis. The general significance of this contraction, and its relation to the changes that take place during the early heterotypic prophase, has been apparently the cause of many confusing accounts and interpretations by many investigators. The massing of the chromatin material at one side of the nuclear cavity of the mother-cell nucleus previous to the formation of the chromosomes of the first heterotypic mitosis was first observed in 1895 by Moore,(18) and he called it synapsis. Sargant,(22) in 1897, reported that she observed the same condition, not only in fixed material, but also in the living pollen mother cell of *Lilium*. Following the discovery of this phenomenon several eminent cytologists have taken a great interest in the study of synapsis in both animals and plants. Among the prominent students of synapsis may be cited the following: Allen,(1) who believes that the fusion of threads and of the chromosomes occurs very early in synapsis; Farmer and Moore,(10) who noted the long duration of this stage, the development and loosening of the spireme, and the relative increase in size of the nuclear cavity in *Lilium*; Cardiff,(4) who states that in the plants studied by him, gravity determines the position that the mass shall take; and Gates,(12) who has published an account on the hybrid of *Oenothera* and pointed out the great reduction in length and the thickening of the spireme during synezesis.

Mottier(19) made a detailed description of synapsis and contradicted the theory advanced by Cardiff; Miss Fraser (11) observed synapsis in *Humaria*, a fungus; Yamanouchi(26) described the synapsis observed in *Nephrodium*, a fern; Davis(6, 7, 8) explains the phenomenon of synapsis as due to general and slow contraction of the reticulum away from the nuclear membrane, a contraction that carries most of the strands toward the center of the nucleus; Gregoire,(14, 15, 16) whose general interpretation does not differ very much from the others, although he believes that the pairing of the maternal and paternal elements takes place during this period of the nuclear activity; Lawsons,(17) who made a critical study of this phase of the heterotypic mitosis, believes that—

> . . . during this phase known as synapsis there is no contraction whatever of the chromatin substance, and this contraction stage has nothing whatever to do with the blending or fussion of maternal and paternal chromatin threads and consequently plays no rôle in the process of chromosome reduction.

In his concluding paragraph he states—

> My interpretation of the phenomenon known as synapsis is simply that it represents a growth period of the nucleus—a condition that is in harmony with the peculiar organization of spore-mother-cells. It is a period during which the increasing karyolymph exerts a great osmotic pressure from within. This pressure results in the extension of the nuclear cavity towards an intercellular space where there is least resistance from the neighboring cells. The chromatin mass is left behind, and its characteristic position at one side of the nuclear membrane is a perfectly natural one.

On the other hand, Gates and Rees have shown by means of a series of measurements in the growth period of *Lactuca* pollen mother cells, that in synezesis the reticulum undergoes contraction to approximately half the diameter of the resting nucleus, and that there is a steady growth of the nucleus. Their findings were corroborated by the writer's observations on *Elodea gigantea*,(20, 21) in which careful measurements on the growing period of the pollen mother cells were also made.

Recently Digby,(9) in her extensive work on *Osmunda*, has indicated that during synezesis the association of the chromatin threads derived from the telophasic split of the chromosomes during the last somatic mitosis is consummated. While Gregoire(14, 15, 16) agrees in the doubling of the spireme during the synezesic contraction, yet he believes that the pairing threads are of paternal and maternal origin. Therefore, these views seem to show that it is during the synezesic contraction when the doubling of the spireme takes place, and that this contraction is apparently the process responsible for the bringing together of the threads. In the case of *Cocos nucifera*, however, it seems that neither of the two interpretations can be applied. The early spireme appears to be distinctly single with a slightly unequal thickening in some places, while in some instances a slight parallelism is observed. But these instances are rare in comparison with the cases in which the spireme is found single. The plausible explanation for this, therefore, is the unequal condensation and contraction of the various threads of the system. During synezesis the delicate univalent threads gradually condense into a relatively short and thick spireme which becomes arranged into as many loops as the bivalent number of chromosomes. From this fact it is evident that in *Cocos nucifera* the chromosomes of the heterotypic mitosis are not formed side by side through the parallel association of two distinct paternal and maternal elements, but are developed from a single spireme, which by segmentation forms thirty-two monovalent chromosomes arranged end to end.

Gates and Rees,(13) in their paper on *Lactuca*, state that—

> The complete loops separate in the segmentation of the spireme. Each bivalent chromosome is thus constituted from the two arms of a loop. This structure condenses greatly, and it appears that in some cases at least the torsion remains. If this account of the formation of the bivalents is true, and we can see no escape from it, the synezesis has no part in bringing about the pairing, and its significance as a unique physiological condition of the nucleus remains entirely obscure.

While the individual loops are gradually separated from each other by process of segmentation, they become short and thick and the free ends do not twist about each other, therefore true "strepsinema" does not occur in *Cocos*. The segments are distinctly single in nature and no sign of fission or splitting can be noticed. As the process of condensation proceeds the spireme passes into a second contraction, the greater portion of the spireme filaments become collected and entangled at one side of the nucleolus.

In the diakinesis stage of *Oenothera franciscana*, Cleland(5) observed the constant and uniform linking together of bivalent chromosomes which, according to him, is—

> . . . a penomenon which can hardly be explained on a parasynaptic basis; and, the most reasonable explanation seems to be that the bivalent chromosomes represent sections of the spireme which occupied such a position in the nucleus that, when the whole system became condensed, the chromosomes found themselves linked in this very definite way.

In the diakinesis of the mother cells of *Cocos nucifera* a more or less similar linking of the chromosomes is observed; this is strong evidence for the end to end arrangement, or telosynaptic interpretation.

SUMMARY

The chromatin reticulum in the resting stage of the pollen mother nucleus of *Cocos nucifera* Linnæus is granular in character and is evenly distributed in the nuclear cavity.

In the early heterotypic prophase the chromatin threads are arranged in a very irregular manner and in them there is a very slight parallelism.

The synezesis is brought about by the contraction of the reticulum, accompanied by a rapid growth of the nucleus. The spireme is formed by an irregular condensation of the chromatin threads and appears at this stage single and unsplit.

Following the long period of synezesic contraction the univalent unsplit spireme gradually spreads throughout the nuclear vacuole and forms loops equal to the gametic number of the chromosomes.

Soon after the spreading of the spireme it passes into a second contraction, and this process is followed by the gradual separation of the loops from one another by segmentation. Each loop consists of two monovalent chromosomes arranged end to end.

While the segments emerge from the second contraction they undergo considerable condensation and become shorter and thicker.

The thickened segments at the early diakinesis stage appear like J's, O's, X's, Y's, and U's, while in the later stages some of them simulate dumb-bell-shaped bodies, and still others, straight and bent rods.

There are sixteen bivalent chromosomes, and in some instances, due to overlapping, there are fifteen. One of the chromosomes is strikingly larger than the others, while three of the chromosomes are smaller.

The multipolar spindle appears after the dissolution of the nuclear membrane and nucleolus.

At the metaphase stage the bivalent chromosomes are arranged more or less evenly and symmetrically in one plane.

All the sixteen bivalent chromosomes split lengthwise at the same time, and as the daughter chromosomes move toward the two poles they take on the V- or the J-shape, with apex toward the poles.

The splitting, or fission, is observed only at the late telophase stage during the formation of the nuclear membranes of the daughter nuclei.

The daughter nuclei resulting from the first division do not pass into anything resembling a typical resting condition. They soon reorganize and prepare for the homœotypic division.

As the nuclear membranes and the nucleoli of the daughter nuclei disappear, the two homœotypic spindles make their appearance simultaneously. The univalent chromosomes proceed to the two equatorial plates and split lengthwise equally to form the four granddaughter nuclei.

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ILLUSTRATIONS

[All figures were drawn with the aid of a camera lucida under the Spencer 1.5 mm. N. A. I. 1.30 apochromatic objective in combination with Carl Zeiss K 20 ocular and Carl Zeiss Orthoskop-Ockular 28x, giving a magnification of 4,100 and 5,500 diameters, respectively. The drawing were reduced to two-thirds in the reproduction.]

PLATE 1. COCOS NUCIFERA LINNÆUS

FIG. 1. A resting nucleus in pollen mother cell, showing a single large nucleolus and the uniformly distributed delicate threads with nodelike chromatic thickening. × 2733.

2. A pollen mother-cell nucleus entering into early prophase; note the irregular thickening of the threads in the reticulum. × 2733.

3. A definite stage of the heterotypic prophase. The reticulum is more defined, consisting of fine threads with irregular thickening and nodelike chromatic granules at the junction of the meshes. × 2733.

4. The beginning of the synezesic contraction and early stage of the spireme; note its irregular thickening. × 2733.

5. A portion of a superficial section of a nucleus entering into synezesic contraction in the same stage as fig. 4. × 3666.

6. A more-definite stage of early synezesis, showing the gradual withdrawal of the early spireme from the nuclear membrane. × 2733.

7. A superficial section of a nucleus in the same stage as fig. 6, showing the unsplit and univalent character of the spireme. × 3666.

8. An early stage of synezesis. The unilateral massing of the chromatin threads is more conspicuous and the meshes are greatly reduced in size. × 2733.

PLATE 2. COCOS NUCIFERA LINNÆUS

FIG. 9. A more-advanced synezesic contraction; the threads and the meshes are drawn into a close mass. A portion of the spireme is extending across the nuclear cavity to the nuclear membrane. × 2733.

10. The appearance of the spireme at the complete synezesic contraction. × 2733.

11. A thin tangential view of the synezesic knot in the same stage as fig. 10, showing the hazy outline and the spiral twisting of the spireme. × 3666.

12. The univalent spireme emerging from the synezesic knot; note the delicate fibers connecting the loops to the nuclear membrane. × 2733.

13. The loosening of the spireme from the synezesic knot and the formation of loops is well underway. The spireme appears more distinct and uniform in character. × 2733.
14. A segment of a superficial section in the same stage as fig. 13. × 3666.
15. An early stage of the hollow spireme; note the loops extending toward the periphery of the nuclear cavity and the well-defined and more-uniform outline of the spireme. × 2733.

PLATE 3. COCOS NUCIFERA LINNÆUS

FIG. 16. A complete hollow spireme. × 2733.
17. A later stage of the hollow spireme; the loops are shortening and the sides of each loop are brought close together while the segmentation begins to take place. × 2733.
18. Slightly older stage than the one shown in fig. 17. The approximation of the sides of each loop and the segmentation are more evident. × 2733.
19. Early stage of the second contraction. × 2733.
20. A complete second contraction. × 2733.
21. The bivalent segments coming out from the second contraction, which are greatly thickened and shortened. × 2733.

PLATE 4. COCOS NUCIFERA LINNÆUS

FIG. 22. The bivalent segments after the second contraction. × 2733.
23. Early dinkinesis stage. The bivalent segments are under the process of condensation and assume different configurations. × 2733.
FIGS. 24 and 25. Slightly older, two similar stages, showing the U-, Y-, J-, O-, and V-shaped chromosomes. × 2733.
26*a* and 26*b*. The two halves of the pollen mother-cell nucleus with sixteen bivalent young chromosomes. × 2733.

PLATE 5. COCOS NUCIFERA LINNÆUS

FIG. 27. A tangential section of the nucleus, a slightly older stage than fig. 26, showing the X-, O-, Y-, and V-shaped chromosomes. × 2733.
28. Three bivalent segments in the same stage as fig. 27 under higher magnification. × 3666.
29. A tangential section of the nucleus showing the appearance of the bivalent chromosomes after a greater condensation; shortening and thickening have taken place. × 2733.
FIGS. 30 and 31. Two diakinetic stages, one closely following the other. The bivalent chromosomes appear as dumb-bell-shaped and as straight and bent rods. × 2733.
32 and 33. Another two successive stages of the late diakinesis, showing the formation of the multipolar spindle fibers. × 2733.
FIG. 34. Spindle fibers becoming bipolars and the bivalent chromosomes are moving toward the equatorial region. × 2733.

PLATE 6. COCOS NUCIFERA LINNÆUS

FIG. 35. A polar view showing the sixteen bivalent chromosomes at the equatorial plate. × 3666.

36. Side view of the metaphase of the first division. × 3666.

37. Typical side view of anaphase in which the daughter chromosomes are mostly V- and J-shaped with apex of V and J attached to the spindle. × 3666.

38. Early telophase of the heterotypic mitosis. × 3666.

39. A somewhat oblique polar view of the same stage as fig. 38. The daughter chromosomes are mostly V-shaped. × 3666.

40. A typical heterotypic telophase, showing the elongation of the daughter chromosomes, their splitting, and the formation of the clear spaces between them. × 3666.

41. A later stage of one of the daughter nuclei in the heterotypic telophase after the appearance of the nuclear membrane. × 3666.

PLATE 7. COCOS NUCIFERA LINNÆUS

FIGS. 42 and 43. Two stages of one of the daughter nuclei, indicating the very irregular shape of the daughter chromosomes apparently going to resolve, but remaining more or less in this condition for a short period and not passing to true resting stage. × 3666.

FIG. 44. A polar view of the daughter chromosomes at the metaphase stage of the homotypic mitosis. × 2733.

FIGS. 45 and 46. Two views of homotypic metaphases. × 2733.

FIG. 47. A homotypic telophase, showing the side view of the future granddaughter nuclei. × 2733.

48. A granddaughter nucleus after the appearance of the nuclear membrane and the nucleolus. × 3666.

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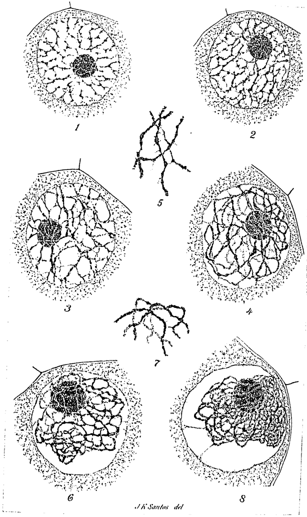

PLATE 1.

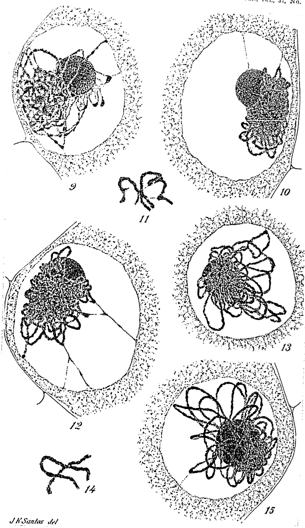

PLATE 2.

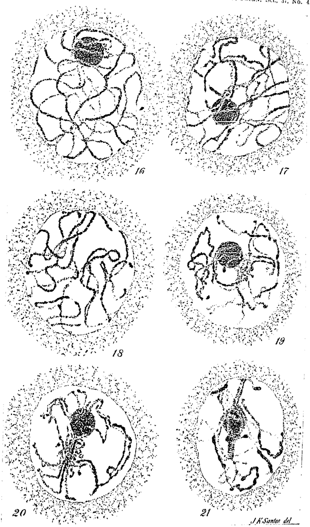

PLATE 3.

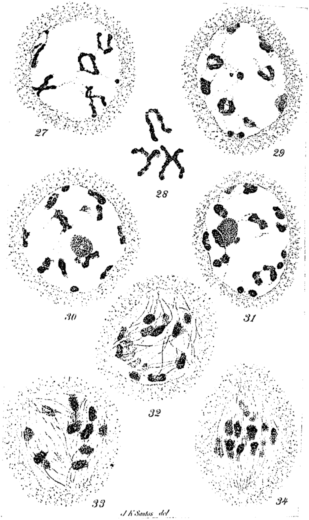

PLATE 5.

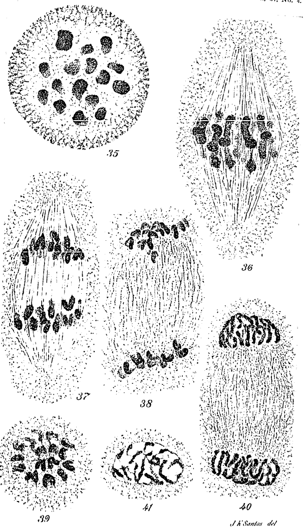

PLATE 6.

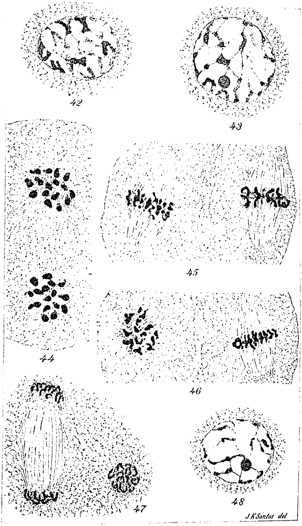

PLATE 7.