THE RELATION OF A PARTICULAR CHROMOSOMAL ELEMENT TO THE DEVELOPMENT OF THE NUCLEOLI IN ZEA MAYS.

By

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With 21 figures in the text and plates VIII—XIV.

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Introduction.

The study described in this paper was undertaken to show that the nucleolus is organized in the telophase by an enlarged, morphologically distinct, deep-staining chromosomal body which appears at a definite position in one chromosome of the monoploid complement of *Zea mays*. It was known that only one nucleolus appeared in each sister nucleus at the telophase of the first nuclear division in the microspore. The microspore contains the monoploid complement of ten chromosomes, one of which possesses the deep-staining body mentioned above. In diploid somatic tissue, two such chromosomes are present. The telophase nuclei in such diploid tissue show two nucleoli. In both the diploid somatic nuclei and the haploid spore, the deep-staining body mentioned above was always observed to be adjacent to the nucleolus. An interchange, produced through X-rays treatment, (ANDERSON, in press) divided this body into two parts, each interchanged chromosome possessing a section. It was found through examination of plants homozygous and heterozygous for this interchange that both sections of this body could function independently to produce a nucleolus.

The nucleolus has been the subject of numerous investigations. Although efforts have been made to attribute a function to it, no satisfactory answer has been forthcoming. It is only in comparatively recent

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years that a definite relationship between the nucleolus and the chromosomes has been established. Although many earlier workers noted the relations of particular chromosomes to the nucleolus (S. NAWASHIN, 1912, 1913; SOROKINE, 1924, 1927, 1929; SENJANINOVA, 1926; BARANOV, 1925, 1926; M. NAWASHIN, 1925; KUHN, 1928, METZ, 1927) the significance of this relationship was not well understood before the work of HEITZ, 1931. In his very illuminating paper, HEITZ pointed out (1) that satellite stalks and secondary constrictions were functionally synonymous (HEITZ called such chromosomes SAT-chromosomes since the stalk of the satellite or the secondary constriction was found to be *sine acido thymonucleinico*), (2) that the nucleolus originated in the telophase at the position of the satellite stalk or secondary constriction, (3) that the number of nucleoli formed in the telophase nuclei depended upon the number of SAT-chromosomes present in the complement, (4) that when two SAT-chromosomes were present in a monoploid complement, distinctive size differences of nucleoli could result, and that this could be correlated with differential functional activities of the two SAT-chromosomes, (5) and that fusion of such nucleoli could occur very early in the telophase. This latter point had been observed by earlier workers in both fixed and living material. It was the belief of HEITZ, however, that the nucleolus was formed by the stalk of the satellite or the secondary constriction. It is the purpose of this paper to indicate that the nucleolus originates not from the stalk or the secondary constriction but from an organized body in the chromosome directly adjacent to the stalk of the satellite. The stalk is very likely produced as the result of the growth of the nucleolus during the telophase. It will also be shown that where two SAT-chromosomes are present in a monoploid complement, the size of the nucleolus developed by each chromosome depends upon the relative functional capacity of the nucleolar organizing bodies in each of the two chromosomes.

The investigations of HEITZ were anticipated slightly by the work of DE MOL. In his observations of the nuclei of polyploid Hyacinths, DE MOL noticed that the maximum number of nucleoli found in the resting nucleus was correlated with the number of monoploid complements present. He also realized that the secondary constriction present in one chromosome of the monoploid complement was definitely related to the nucleolus. However, he did not emphasize the significance of this relationship.

Since the appearance of the paper of HEITZ, other investigators have reported the relationship of particular chromosomes of the complement to the nucleolus (SAX, 1932; SMITH, 1933; KAUFMANN 1933; GEITLER, 1932; and also, again, HEITZ, 1933). The association of a particular chromosome of a complement with the nucleolus is well established. The presence of a definite deep-staining body in the chromo-

some at the position of union with the nucleolus has been mentioned by STEVENS (1905), BARANOV (1925), DAWYDOV (1930) and SAX (1932).

The methods used were as follows: For a study of the meiotic mitoses and the microspore nucleolar divisions, the method of acetocarmin smears described in earlier papers (McCLINTOCK, 1929, 1931) was utilized. For a study of the somatic tissue in root meristems, chrom-acetic-formalin was used as a killer and acid fuchsin and methyl green were used as stains. The outline drawings were made with the aid of a camera lucida. Where possible, photomicrographs have been used as illustrations. The term "figure" will be employed to designate the text figures, the term "photo." to designate the photographs in the plates.

Structure of the satellited chromosome.

The structure of the satellited chromosome (chromosome 6 of the monoploid complement) in *Zea mays* is best seen at the mid-prophase of meiosis (pachytene)[1]. This is shown photographically in fig. 1. For descriptive purposes it has been divided into six parts. Part *1*, fig. 1, is the satellite proper. It is usually composed of four distinct chromomeres. The basal chromomere is adjacent to the nucleolus. A thin, practically colorless thread or ribbon, part *2* (not clear in this photograph), running across the surface of the nucleolus joins the basal chromomere of the satellite with a large deep-staining body, part *3*, (and arrow photo. 10, plate IX) which is conjoined to the nucleolus. The structure of this deep-staining body is sometimes definitely reticulate. It will be shown in the following pages that this particular body, possessed only by chromosome 6 of the normal maize complement, is responsible for the orderly organization of the telophase nucleoli. In this paper it will be referred to as the nucleolar-organizing body. This conspicuous body is followed by a chromatic thread, part *4*, composed of chromomeres, which extends to the spindle fiber attachment region, part *5*. Part *6* is the long arm of the satellited chromosome. Besides the distinctive features of chromosome 6 already described it may possess, toward the end of the long arm, one or two knobs. These knobs have characteristic physical features and definite locations in the chromosome. It has been pointed out previously (CREIGHTON and McCLINTOCK, 1931) that such knobs can be followed through generations with the same precision as genes. Whether a particular satellited chromosome (chromosome 6) has none, one or two knobs depends, therefore, upon the particular culture which is used.

At somatic telophases, in diploid plants with two normal satellited chromosomes, two nucleoli, usually of similar size[2], are formed. Frequently, fusion of the two nucleoli takes place in the early telophase to form one. Thus, in diploids, somatic nuclei are characterized by one or two nucleoli.

[1] For variations in the types of satellited chromosomes, see page 307.

[2] For variations in size see page 316.

The attachment of the satellited chromosome to the nucleolus in somatic prophases is similar to that described for the microspore stage given below.

The microspore is very convenient for such a study. Only one chromosomal complement is present. Each chromosomal complement is isolated in a separate spore. The methods allow the prophase figures to be somewhat flattened thus affording a better view of the satellited chromosome.

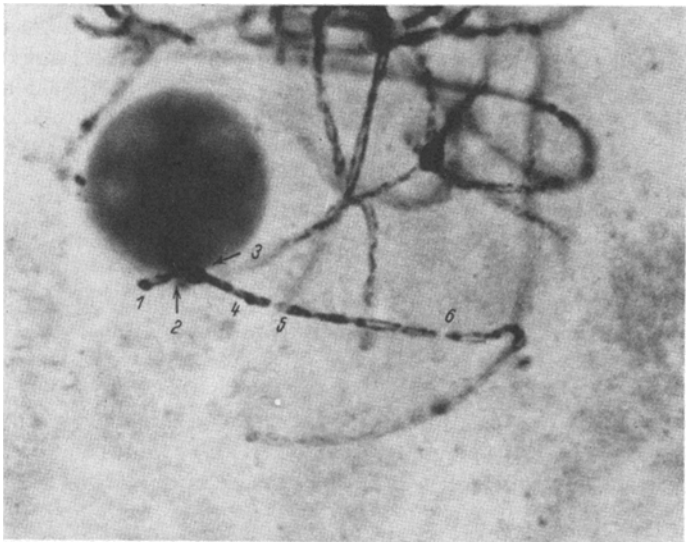

Fig. 1. Photomicrograph of two homologously associated chromosomes 6 (satellited chromosome) in the mid-prophase of meiosis showing the relationship to the nucleolus. The parts of the chromosome have been numbered; *1*. the satellite proper; *2*. the region joining the satellite to the, *3*, deep-staining nucleolar-organizing body; *4*. the chromatic thread of the short arm; *5*, the spindle fiber attachment region; *6*. the chromatic thread of the long arm. Magnification approx. 1400 ×.

Large numbers of prophase figures are available. In the prophase of a microspore with a normal monoploid complement there are ten identifiable chromosomes. There is but one nucleolus. The satellited chromosome is attached to the nucleolus, fig. 17. The attachment of this chromosome to the nucleolus is similar to that in the prophase of meiosis with the exception that the satellite, with its basal part adjacent to the nucleolar surface, is usually some distance removed from the nucleolar-organizing body. In most cases it is not possible to see a connection joining the satellite with the nucleolar-organizing body. In the drawings, therefore, a connection has been indicated by a dotted line. As the prophase approaches the metaphase conditions, the nucleolus decreases in size. As it does so the satellite begins to approach the nucleolar-organizing body. However, before this is concluded, the rapid dissolution of the

nucleolus completely releases the satellited chromosome. The thread joining the satellite with the nucleolar-organizing body is then clearly visible. The length of this thread varies greatly in different figures and appears to depend upon the distance the satellite was removed from the nucleolar-organizing body in the previous prophase and also, upon the stage at which the satellited chromosome is released from the nucleolus in the late prophase. This variation in length of the thread joining the satellite to the nucleolar-organizing body is visible in the metaphase chromosome. The microspore is particularly useful for telophase studies since the two nuclei in a spore are known to be sister nuclei. A single nucleolus is formed in each sister nucleus. They lie in corresponding positions.

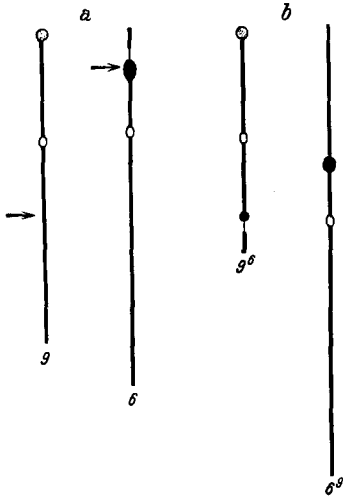

Fig. 2. Diagram of the chromosomes involved in the interchange. *a* chromosomes 6 and 9. The short arm of chromsome 9 terminates in a knob. The breaks in the chromosome connected by slighthly bulging light lines indicate the position of the spindle fiber attachment region. The arrows point to the position in each chromosome where the interchange occurred. *b* The two chromosomes resulting from the interchange. Chromosome 9^6 has the small section of the nucleolar organizing body; chromsome 6^9 has the larger section of the nucleolar-organizing body.

Description of interchange.

Proof that the deep-staining body of chromosome 6 which lies adjacent to the nucleolus is associated with the orderly organization of the telophase nucleoli was obtained from an interchange which divided this region into two parts. Each part was found capable of organizing a separate nucleolus.

The interchange involved chromosome 9 and chromosome 6 (satellited chromosome). Chromosome 9, fig. 2, is characterized by the relative lengths of its two arms. The long arm is almost twice the length of the short arm when measured in the mid-prophase of meiosis. In most cultures, the end of the short arm possesses a deep-staining knob. Chromosome 6 has been described on page 296. The interchange occurred at the position of the arrows, *a*, fig. 2, to produce the two chromosomes 9^6 and 6^9, *b*, fig. 2. From the diagram it can be seen that the interchange divided the deep-staining body adjacent to the nucleolus into two unequal parts. Chromosome 9 lost two-thirds of its long arm and received in its place a small section of the deep-staining body of chromosome 6, plus the satellite of chromosome 6. Since it retained all of the short arm, the spindle fiber attachment region and one-third of the long arm of chromosome 9, this new chromosome has been designated chromosome 9^6. Chromosome 6, on the

other hand, retained a large portion of the deepstaining body. The segment from the long arm of chromosome 9 attached itself at its broken end to the broken end of the deep-staining body of chromosome 6. The resulting chromosome has been designated chromosome 6^9 since it possesses the long arm, the spindle fiber attachment region and most of the short arm of chromosome 6.

Somatic and meiotic nucleolar conditions in plants homozygous for the interchange.

It has been stated on page 294 that two nucleoli develop in the somatic telophase of normal diploid plants. There are two chromosomes 6 in each somatic nucleus. The two nucleoli are developed in conjunction

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 3. Outline sketch of a nucleus of a root-tip cell in a plant trisomic for chromosome 6. Three nucleoli of approximately similar size are present.

Fig. 4. Outline sketch of a nucleus of a root-tip cell in a plant heterozygous for the interchange. There are two large nucleoli and one small nucleolus. The two large nucleoli resulted from the functioning of the nucleolar-organizing elements of chromosomes 6 and 9^6, the small nucleolus from the functioning of the nucleolar-organizing elements of chromosome 6^9.

Fig. 5. Outline sketch of a nucleus from a root-tip cell in a plant homozygous for the interchange. The two large nucleoli were formed through the functioning of the nucleolar-organizing elements of the two chromosomes 9^6; the two small nucleoli were formed through the functioning of the nucleolar-organizing elements of the two chromosomes 6^9. (The small nucleolus to the left has been displaced in the drawing since it was lying in the same plane as the large nucleolus to the left.)

with the nucleolar-organizing bodies of each chromosome 6, respectively. The union between the nucleolar-organizing body and the nucleolus is retained through the resting period and into late prophase of the next nuclear division.

The nucleolar condition in diploid plants homozygous for the interchange is strikingly different from that observed in normal diploids. The maximum number of nucleoli present in a nucleus is not two, as in normal diploids, but four. Of these four nucleoli, two are large and two are small, fig. 5.[1] The relation between these four nucleoli and the interchanged chromosomes will be brought out in the description which follows. Plants homozygous for the interchange possess two each of chromosomes 9^6 and 6^9. Homologous associations at the mid-prophase of meiosis gave three main types of figures. The first type is shown in photos. 1, a and b, and 2 a, b and c, and sketches of same, fig. 6 and 7,

[1] Due to fusions between several or all of these four nucleoli other nucleolar conditions, with reference to number and size were observed.

respectively. There is one large nucleolus. Chromosome 9^6 bivalent is attached to the nucleolus by its segment of the nucleolar-organizing body. The satellite is usually a short distance removed from this body and joined to it by an almost unstained thread or ribbon which is likewise attached to the nucleolus. This association is similar to that described

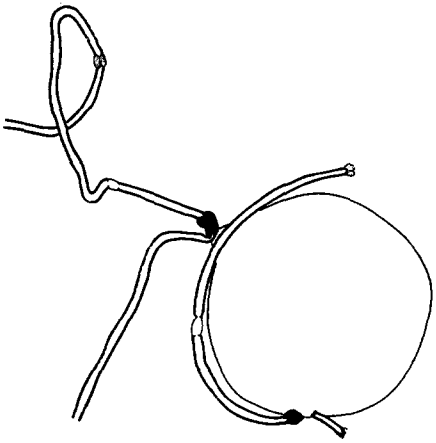

Fig. 6. Outline sketch of a pachytene configuration in a plant homozygous for the interchange. See photograph of the same, photo. 1a and b. Both chromsome pairs are attached to the nucleolus by their deep-staining nucleolar-organizing bodies. Chromosome pair 6^s is to the left, chromosome pair 9^s to the right. The spindle fiber attachment region is indicated by the slightly bulging light lines. This region of chromosome 9^s is clearly visible in the photograph.

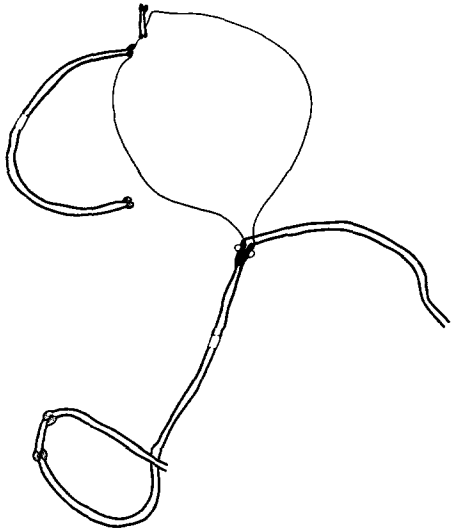

Fig. 7. Outline sketch of a pachytene configuration in a plant homozygous for the interchange. See photograph of same, photo. 2a, b and c. Chromosome 9^s, upper left, chromosome 6^s below. Both are attached to the single nucleolus by their nucleolar-organizing bodies.

for the normal chromosome 6, page 296. Chromosome 6^9 is also attached to the nucleolus by its section of the nucleolar-organizing body. However, in this case, the interchanged piece belonging to the long arm of chromosome 9 is almost always joined directly to the deep-staining segment of the nucleolar-organizing body of chromosome 6^9, i. e., it is not removed from this body by an unstained thread. This can be seen in photos. 1 and 2. In these photos. the difference in size between the two segments of the nucleolar organizing body possessed by each interchanged chromosome is evident.

The second type of configuration is illustrated in photos. 3 and 4. These cells were photographed at a lower magnification than those shown in photos. 1 and 2. The two interchanged chromosomes with their respective nucleoli, shown in photo. 3, are sketched in fig. 8. In these sporocytes there are two nucleoli, one large and one small. The chromosome bivalent 9^6 is always associated with the large nucleolus, the chromosome bivalent 6^9 with the small nucleolus. It is necessary to emphasize this correlation since it is a part of the evidence which has lead to the

conclusion that the small segment of the nucleolar-organizing body possessed by chromosome 9^6 produces a large nucleolus whereas the large segment of the nucleolar-organizing body possessed by chromosome 6^9 produces a small nucleolus.

A third type of configuration is sometimes found. In these figures the nucleolar-organizing bodies of each of the four chromosomes are

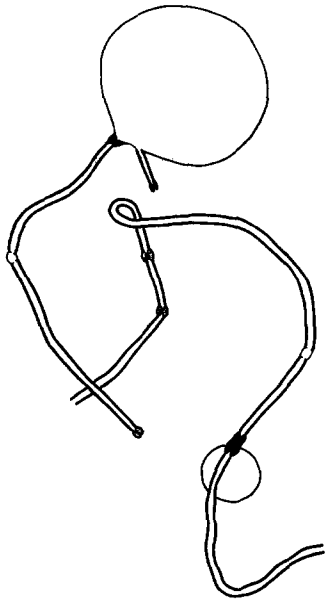

Fig. 8. Outline sketch of a pachytene configuration of a plant homozygous for the interchange. See photograph of same, photo. 3. Chromosome 9^6 is associated with the larger nucleolus; chromosome 6^9 with the smaller nucleolus.

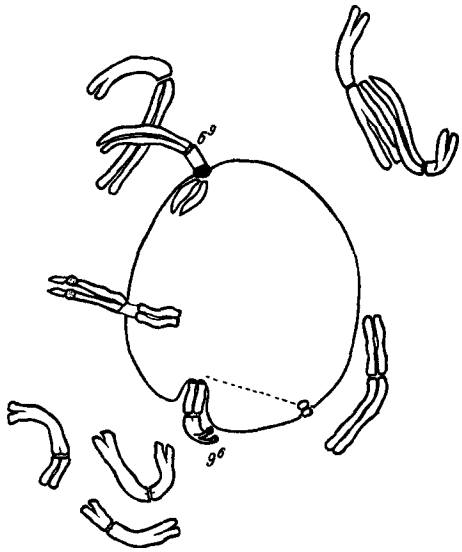

Fig. 9. Outline sketch of a prophase chromosome complement in the microspore of a plant homozygous for the interchange. See photograph of same, photo. 28. Chromosomes 6^9 and 9^6 are associated with the single nucleolus. Note that the satellite of chromosome 9^6 is distantly removed from the small but deeply-staining nucleolar-organizing body of this chromosome and that the translocated segment of chromosome 6^9 lies close to its deep-staining nucleolar-organizing body. The "breaks" in chromosomes are the achromatic spindle fiber attachment regions.

together. The resulting configuration resembles those found in plants heterozygous for the interchange.

The microspores of these plants were studied. The prophase complements were of two kinds, those with one nucleolus and those with two nucleoli. In microspores with one nucleolus, chromosomes 9^6 and 6^9 are both attached to the single nucleolus, fig. 9 and photograph of same, photo. 28. The satellite on chromosome 9^6 is always some distance removed from the small segment of the nucleolar-organizing body of this chromosome (dotted line, fig. 9). Chromosome 6^9 is attached to the nucleolus by its section of the nucleolar-organizing body. The section of the long arm of chromosome 9 in chromosome 6^9 usually lies close to the nucleolar-organizing body of this chromosome, fig. 9. Occasionally,

however, it is removed a short distance from the nucleolar-organizing body. In this case, its basal part remains adjacent to the nucleolus. In an occasional spore chromosome 6^9 appeared entirely free from the nucleolus. Chromosome 6^9 in these spores can be readily identified by its deep-staining knob and also its deep-staining nucleolar-organizing body. In these cases, the section of the long arm of chromosome 9 possessed by chromosome 6^9 is always directly adjacent to the nucleolar-organizing body.

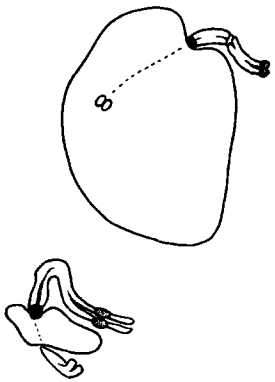

Fig. 10. Chromosomes 6^9 and 9^6 from a prophase of a microspore in a plant homozygous for the interchange. Chromosome 9^6 is associated with the larger nucleolus. The satellite is some distance removed from the nucleolar-organizing body of this chromosome. Chromosome 6^9 is associated with the smaller nucleolus. The translocated segment is removed from the nucleolar-organizing body of this chromosome during the growth of the small nucleolus.

The second type of spore possessed two nucleoli, one large and one small, fig. 10. In this case, chromosome 9^6 is always associated with the large nucleolus, chromosome 6^9 with the small nucleolus.

The metaphase chromosome complement in a spore of a plant homozygous for the interchange is shown in fig. 11 and photograph of same, photo. 31. It will be noted that there is a small satellited chromosome and a longer chromosome with two "constrictions". The small satellited chromosome is chromosome 9^6, the longer chromosome with two "constrictions" is chromosome 6^9. The distal "constriction" on chromosome 6^9 is associated with the previous nucleolar attachment, whereas the more median "constriction" is the spindle fiber attachment region.

The telophases of the first division of the nucleus in the microspore are characterized by the production of two nucleoli in each sister nucleus, one large and one small. The positions of the nucleoli correspond in the two nuclei, photos. 18, 19 and 20. Fusion of the two nucleoli frequently takes place very early in the telophase, upper nucleus photo. 20. The difference in size between the two nucleoli is quite striking in most telophase nuclei. However, there is some variation. In a few spores the two nucleoli in the telophase nucleus were more nearly the same size. This however, is rare and will be considered on page 316.

Examination of nuclei of plants homozygous for the interchange in diploid somatic tissue, in meiosis and in the microspores has been particulary instructive (1) in indicating that the interchange divided the nucleolar-organizing body into two unequal parts, (2) in showing the relative amounts of the nucleolar-organizing body that each chromosome possessed, (3) in proving that each section of the divided nucleolar-organizing body can function to produce a nucleolus and (4) in showing

that the nucleoli produced by each section of the nucleolar-organizing body bear a definite size relationship to one another: the small section of the nucleolar-organizing body carried by chromosome 9^6 produces a large nucleolus whereas, the large section of the nucleolar-organizing body carried by chromosome 6^9 produces, in contrast, a small nucleolus. The following interpretation is placed upon this latter observation. The usual satellited chromosome (chromosome 6) possesses a large, somewhat elongated nucleolar-organizing body (*3*, fig. 1), which is confluent with the nucleolus mainly at one region. The rest of the nucleolar-organizing body frequently is free from the nucleolus. Examination of plants homozygous for the interchange (and also those heterozygous for the interchange) indicates that the point of interchange is near that end of the nucleolar-organizing body which is directly adjacent to the nucleolus. In consequence, chromosome 9^6 obtained the small section of the nucleolar organizing body which in the normal chromosome 6 is usually confluent with the nucleolus, and chromosome 6^9 retained that part of the nucleolar-organizing body of chromosome 6 which, in the normal chromosome 6, is usually free from the nucleolus. This evidence suggests that the functional capacities of different sections of the nucleolar-organizing body vary, i. e. that certain regions may possess greater functional capacities than other regions. On this basis, it can be assumed that in the normal chromosome 6 the distal region (farthest from the spindle fiber attachment region) has a greater functional capacity than the proximal region. That this differential capacity for functioning is a matter of speed reaction will be brought out later (page 316).

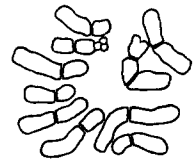

Fig. 11. Metaphase chromosomes in the microspore of a plant homozygous for the interchange. See photograph of the same, photo. 31. The small chromosome with the satellite (upper left) is chromosome 9^s; the chromosome with the secondary "constriction" (to the right of chromosome 9^s) is chromosome 6^9.

Nucleolar conditions in the microspores of plants heterozygous for the interchange.

The main facts regarding the relation of a particular body in a particular chromosome to the origin of a nucleolus could be obtained through observations of plants homozygous for the interchange.

Examination of plants heterozygous for the interchange (possessing one each of chromosomes 6, 9, 6^9 and 9^6) has widened the point of view and has added considerable to the evidence already obtained. The maximum number of nucleoli observed in the somatic nuclei of these plants was three, two large and one small, Fig. 4. The evidence obtained from plants homozygous for the interchange would suggest that the two large nucleoli develop in conjunction with the nucleolar-forming elements of chromosomes 6 and 9^6, whereas the small nucleolus develops

in conjunction with the nucleolar-organizing element of chromosome 6^9. However, the primary interest with reference to plants heterozygous for the interchange concerns the nucleolar conditions of the microspores. It was anticipated that distributions at the first meiotic anaphase (*A I*) of the four chromosomes involved in the interchange complex would produce various types of spores each with a definite nucleolar condition. The nucleolar condition of two of the spore types, those with the two normal chromosomes and those with the two interchanged chromosomes, could be anticipated, but the nucleolar conditions of the other types of spores had to be determined. In consequence, it was necessary to study the stages from early prophase of meiosis to the pollen itself. Through a study of the first meiotic anaphase it was possible to anticipate, to some extent, the types of spores that would be formed. By means of direct examination of the spores themselves, it was possible to classify and thus to verify the conclusions derived from the study of *A I* distributions. Through this study, some interesting and unanticipated results were obtained with reference to the development of the nucleolus.

A. Chromosome distribution in the first meiotic anaphase.

At the mid-prophase of meiosis in plants heterozygous for the interchange, homologous association of the four chromosomes produces a "cross-shaped" configuration, fig. 12 and 13 and photographs of same, photos. 5 and 6, respectively. In photo. 6 only the region close to the center of the "cross" is shown. The center of the "cross" is within the nucleolar-organizing body. An early diplotene stage is shown in photo. 9. Chromosome 9 possessed a terminal knob on the end of the short arm, chromosome 9^6 was knobless. This heterozygosity is visible (upper right region of the photograph). At diakinesis, both rings and chains were observed. A ring at diakinesis is shown in photo. 12. There is a small nucleolus attached at the region of the nucleolar organizing body chromosome 6^9. In the chain configurations, the break nearly always involves a separation of the two satellites of chromosomes 9^6 and 6, photo. 11^1. The order of the chromosomes in the chain is therefore, 6, 6^9, 9 and 9^6 with the two satellites of chromosomes 6 and 9^6 completely unassociated.

[1] Some of the chain formation in diakinesis can undoubtedly be attributed to lack of homologous associations in the previous prophases between the two satellites of chromosome 6 and 9^6. It has been shown in a previous paper (McClintock, 1933) that non-homologous association in prophase usually results in lack of association in diakinesis. In plants heterozygous for the interchange a large number of non-homologous associations were observed. These were mainly confined to the regions about the nucleolar-organizing bodies and in consequence frequently involved the satellites. Two cases of extensive non-homologous associations which did not, however, involve the two satellites, are shown in photos. 7 and 8, and sketches of same, fig. 14 and 15.

Counts of anaphase *I* distributions were attempted to obtain an indication of the type of segregation which occurrs in this interchange. Rings and chains are easily observed at metaphase and anaphase. The simple ring configurations showed two main types of distributions, "alternate" distributions, photo. 13, (arrow) and "adjacent" distributions, photo. 15 (center of figure). The distribution of the simple chain configurations were also both alternate, photo. 16 (extreme left) and adjacent, photo. 17 (extreme left). Here, however, a striking correlation between the position of the chromosomes in the chain and the type of adjacent distribution was observed. In nearly all cases, the chromosomes were arranged on the plate so that the two chromosomes with unassociated ends were passing to the same pole. Since diakinesis figures showed that a chain was produced by a separation of the two satellited ends of chromosomes 6 and 9^6, it is clear that in chain configurations the adjacent distributions are mostly of one kind. Corroborative evidence was available at *M. I.* in the observations of the satellites themselves at the ends of the chain configurations, photos. 14 a and b. (For explanation of the satellite in b, see page 307).

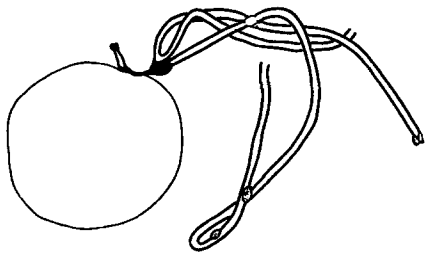

Fig. 12. Outline sketch of a pachytene configuration in a plant heterozygous for the interchange. See photograph of same, photo 5. Association of homologous parts of chromosomes 6, 9, 6^9 and 9^6 results in the production of a "cross-shaped" configuration with the nucleolar-organizing body forming the center of the cross.

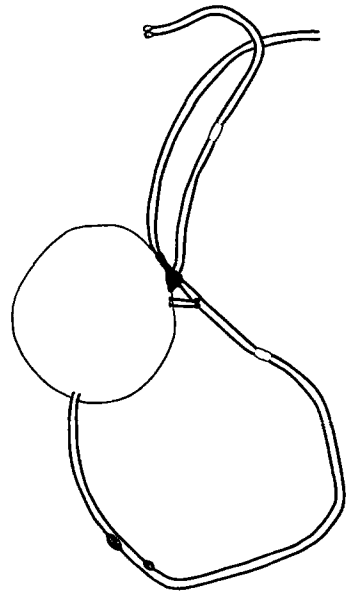

Fig. 13. Same type of configuration shown in fig. 12. A photograph of this configuration showing the region about the nucleolus is given in photo 6.

Barring crossing-over between the spindle fiber attachment region and the position of the interchange, and assuming reductional distributions of the spindle fiber attachment region in *I*, alternate distributions of the chromosomes in the simple chain configurations will give rise to a quartet of spores two with normal chromosome complements and two with interchanged chromosome complements, *a* and *b*, fig. 2. Adjacent

distributions will give rise to a quartet of spores, two with chromosomes 9 and 6^9 and two with chromosomes 6 and 9^6. A cross-over of the type mentioned above will give rise to a quartet of spores possessing one each of the four types of spores. If the distribution in the ring configurations is similar to that in the chain configurations, i. e., only one type of adjacent distribution, only four types of spores will be formed. In making the counts the ring configurations were divided into three classes, those with alternate distributions, those with adjacent distributions and those in which the distribution of the individual chromosomes of the ring could not be stated with certainty. The chain configurations were much easier to observe. Here, also, the configurations were classified into alternate, adjacent and uncertain distributions. The uncertain class was composed in many cases of chain configurations in which there was a chiasma between the spindle fiber attachment region and the position of the interchange. It was not possible in these cases to determine a simple alternate or adjacent distribution. Besides the rings and chains, the number of sporocytes were recorded in which it was not possible to determine whether a ring or a chain configuration was present. This was due mainly to a crowding of the chromosomes on the plate so that the chromosomes involved in the interchange were not detectable.

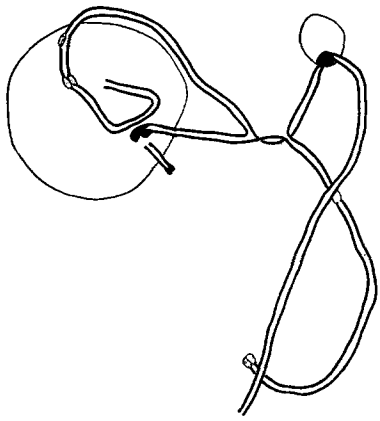

Fig. 14. Outline sketch of a pachytene configuration in a plant heterozygous for the interchange. See photograph of same, photo. 7. As stated in the text, chromosomes 6 and 9^s produce large nucleoli (fused in this figure) whereas chromosome 6^s produces a small nucleolus. Since the small nucleolus of chromosome 6^s has not fused with the nucleoli of chromosomes 6 and 9^s, extensive non-homologous association has resulted in the region between the two nucleoli. The spindle fiber attachment region of chromosomes 6 and 6^s could not be clearly seen in this figure.

In all plants examined there was a small percent of aberrant configurations and distributions. The aberrant configurations consisted mainly of metaphase figures with (1) ten bivalents, or (2) eight bivalents plus a configuration of three, plus a univalent. The aberrant distributions mainly involved (1) adjacent distributions in which chromosomes 6 and 6^9 were passing toward one pole and chromosome 9 and 9^6 toward the opposite pole and (2) distributions of three chromosomes to one pole and one to the opposite pole. Anaphase counts were made to determine the percentage of 11—9 distributions. Of the 384 anaphase figures recorded, 11 were definitely of this type. The other 373 were 10—10 distributions. The aberrant distributions have not been

recorded in the tables because of their small percentage and their heterogeneity.

In making counts of the ring and chain configurations at *M I* and *A I* a marked difference in the percentage of rings to chains was found among the different plants. This difference has been correlated with the presence of a modified satellite on the normal chromosome 6 which results in frequent lack of prophase association or, instead, the presence of non-homologous association between the satellites of chromosomes 9^6 and of chromosome 6. The modified satellite on chromosome 6 is very large with its basal part deeply pycnotic [1]. The difference in the appearance of the normal satellite and the modified satellite can best be observed in the late prophase of the microspore. Such a distinction is illustrated in photos. 34a and b, two photographs of the same microspore showing both the normal and the modified satellite. An outline sketch of these two satellited chromosomes is given in fig. 16. The small satellite is associated with chromosome 9^6 (left), the large satellite with chromosome 6. In photos. 32, 33 and 35, the large modified satellite (arrows) in other microspores are illustrated and can likewise be compared with a normal satellite shown in photo. 28. The distinction in size between these two sa-

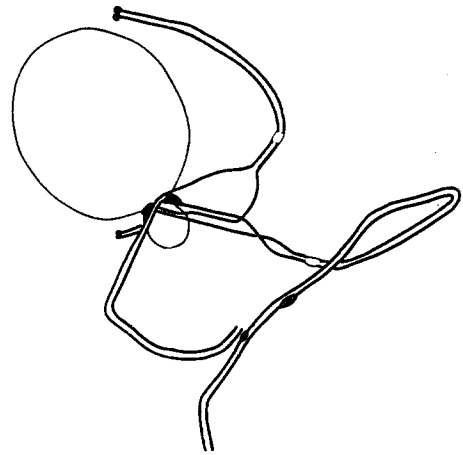

Fig. 15. Outline sketch of a pachytene configuration of a plant heterozygous for the interchange. See photograph of same, photo. 8. Note the non-homologous associations involving chromosomes 6, 6^9 and 9^6 in the section to the right of the nucleolar-organizing bodies.

[1] It has been pointed out, page 303, that in the usual satellited chromosome the position in the large deep-staining nucleolar-organizing body at which the nucleolus is mainly developed is near the distal end of this organ. In certain strains of maize, the position of main activity of the nucleolar-organizing body is nearer the center or even near the proximal end. When the position of greatest functional activity is nearest the proximal end, the formation and growth of the nucleolus at this particular region results in the main part of the nucleolar-organizing body being included in the satellite. Since the nucleolar-organizing body is large and somewhat pycnotic, and contracts in linear dimensions much less than other parts of the chromosomes, the resulting satellite at late prophase and metaphase is very much larger than the usual satellite. This type of chromosome 6 was present in the plants showing a low percentage of ring configurations and a high percentage of chain configurations. It is obvious therefore, that relatively simple heteromorphisms in maize can materially alter the metaphase morphologies.

tellites is very evident at diakinesis and sometimes even at *M I*, photos. 14 a and b. In photo. 14a the arrow points to the small satellite of chromosome 9^6; in photo. 14b the arrow points to the large satellite of chromosome 6.

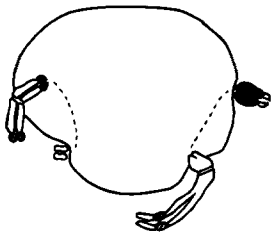

Fig. 16. Chromosomes 6 and 9^6 in the prophase of an 11-chromosome microspore from a plant heterozygous for the interchange. Chromosome 9^6 is the extra chromosome. Chromosome 6, right, has the modified, "large" satellite; chromosome 9^6 the small "normal" satellite. See photograph of same, photos. 34a and b.

The frequently observed lack of prophase association between the two heteromorphic satellites or the substitution of non-homologous associations involving these regions is believed to be the cause of the great increase of chains vs. ring configurations in these plants.

Counts of the anaphase *I* distributions from plants with homomorphic satellites on chromosomes 6 and 9^6 are given in table 1. Counts from plants with heteromorphic satellites are given in table 2. It is realized that the counts are accurate only for the simple

Table 1.

Rings			Chains			Uncertain configurations
Alternate	Adjacent	Uncertain	Alternate	Adjacent	Uncertain	
4	4	10	8	3	3	1
13	27	23	26	21	9	13
41	48	33	42	42	10	36
31	48	19	67	64	17	7
3	11	6	20	23	6	2
30	40	15	44	39	12	20
7	6	1	9	7	6	3
129	184	107	216	199	63	82

420 Rings, 478 Chains, 82 Uncertain.

Table 2.

Rings			Chains			Uncertain configurations
Alternate	Adjacent	Uncertain	Alternate	Adjacent	Uncertain	
5	10	9	41	43	10	27
3	3	0	14	12	0	5
7	12	8	31	35	5	37
0	1	1	0	2	0	0
8	12	12	94	78	17	40
3	5	7	52	38	24	9
16	13	4	34	42	15	15
2	7	1	10	11	5	4
3	10	5	26	20	9	13
1	4	1	24	13	5	3
2	3	0	8	13	7	12
5	11	5	80	94	52	19
55	91	53	414	401	149	184

199 Rings, 964 Chains, 184 Uncertain.

types of ring and chain configurations and that it is not possible to state the percentage of the spore types which will result from such distributions. They serve merely as an indication. Among the ring configurations, in both tables, the number of recorded adjacent distributions was greater than the number of alternate distributions. This is believed to be due, in part, to the greater difficulty in determining alternate distributions in ring configurations. In chain configurations, on the other hand, this difficulty was not present and the proportion of the recorded alternate and adjacent configurations are more nearly equal.

B. Types of spores produced in plants heterozygous for the interchange.

On the basis of the observed anaphase *I* distributions, the chromosome constitution of the four main types of spores can be classified as follows: In all cases, it is assumed that the other eight chromosomes of the monoploid set of ten are present.

Spore	type	1	with	chromosomes	9	and	6
,,	,,	2	,,	,,	9^6	,,	6^9
,,	,,	3	,,	,,	9	,,	6^9
,,	,,	4	,,	,,	6	,,	9^6

In addition to these four types of spores there should be a few 11-chromosome and 9-chromosome spores of the following constitutions:

Spore	type	5	with	chromosomes	9, 6	and	6^9
,,	,,	6	,,	,,	9, 6	,,	9^6
,,	,,	7	,,	,,	$9^6, 6^9$	,,	6
,,	,,	8	,,	,,	$9^6, 6^9$	,,	9
,,	,,	9	chromosome		9		
,,	,,	10	,,		6		
,,	,,	11	,,		6^9		
,,	,,	12	,,		9^6		

The adjacent distributions which are not of the usual kind should give spores with the following chromosome constitutions with respect to the chromosomes involved in the interchange:

Spore	type	13	with	chromosomes	6	and	6^9
,,	,,	14	,,	,,	9	,,	9^6

Identification of the chromosomal constitution of a particular microspore in the late prophase of its first nuclear division was comparatively easy for most ot the spore types. By means of counts of the spore types in anthers of all stages of maturity it was possible to draw the following general conclusions. Most of the spores were of types 1 to 4. Of these four types of spores the rate of nuclear division of spore types 1 and 2, with the full genome complement, was in advance of spore type 3, which, in turn, was in advance of spore type 4. Spore types 5 to 14 were observed relatively infrequently. The nuclei of spore types 5 to 8 were somewhat

slow in their rate of division and the nuclei of spore types 9 to 14 were very slow in their division rates.[1]

In this paper I shall confine my description to the nucleolar condition of the four main types of spores.[2]

Type 1 spores are normal in all respects. They possess the ten chromosomes of the monoploid complement. There is but one nucleolus in

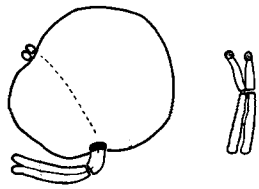

Fig. 17. Chromosomes 6 and 9 in a prophase of a type-1 spore. Chromosome 6 is associated with the nucleolus. The satellite is distantly removed from the nucleolar-organizing body. Chromosome 9 is free from nucleolar associations.

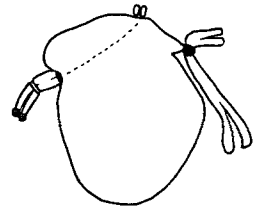

Fig. 18. The nucleolar associations of chromosomes 6^s and 9^s in a prophase of a type-2 microspore. The satellite of chromosome 9^s (left) is distantly removed from the nucleolar-organizing body of this chromosome. The translocated piece of chromosome 6^s (right) is adjacent to the nucleolar-organizing body of this chromosome.

each nucleus and chromosome 6 is associated with it in the manner described on page 297, fig. 17. Type-2 spores, with the two interchanged

[1] In connection with the rate of nuclear division in the different types of spores the following interesting observation was made. In one particular culture the rate of nuclear division among the type-2 spore, with the two interchanged chromosomes, was somewhat in advance of those of spore type 1 although, theoretically, both contained a full genome complement. In this culture it is possible that growth factors, associated with one or both of the interchanged chromosomes were responsible for the variation in rate of division.

[2] With reference to spore types 5 to 14 only a brief mention will be made. Prophase figures of spores with 11-chromosomes were relatively infrequent. In spore types 5, 6 and 8, two recognizable chromosomes were associated with the nucleolus. In spore type 7, three recognizable chromosomes were attached to the nucleolus. Theoretically, spore types 5 to 8 should show, occasionally, two (in spore types 5, 6 and 8) or three (in spore type 7) nucleoli in their prophase nuclei. Since fusion of several nucleoli to form one is most usual, the finding of spores with two or three separate nucleoli should be very infrequent. In the comparatively few 11-chromosomes spores observed only one nucleolus (resulting from previous fusions) was present. As will be described, the telophase nucleolar condition is to a certain extent an indication of the chromosomal constitution of the spore. Several spores in telophase were found in which each sister nucleus possessed three nucleoli, two large and one small, in comparable positions within each nucleus. Such a spore is shown in photo. 22. The presence of two large nucleoli (lower) and one small nucleolus (upper) suggests that the chromosome complement of this spore was of type 7. In the relatively few 9-chromosome spores observed it was not always possible to determine the exact chromosomal constitution since the chromosomes were often distorted in appearance. This same distortion was noticed in spore types 13 and 14.

chromosomes and full genome complement, are similar in all details to those described for the homozygous interchange, fig. 18. See also, prophase fig. 9 and 10 and telophase photos. 28, 29 and 31. Type-3 spores, with chromosomes 9 and 6^9, are deficient for the satellite of chromosome 6 and possess a duplication of two-thirds of the long arm of chromosome 9. A nucleolar-organizing body is carried only by chromosome 6^9. It should be recalled that this is but a segment of the normal nucleolar-organizing body of chromosome 6. Also, it should be recalled that when this chromosome is in the same nucleus with chromosome 9^6 or with both chromosomes 6 and 9^6, its nucleolar-organizing body produces a *small* nucleolus in contrast to those produced by chromosomes 6 and 9^6. However, when present alone, as in spore type 3, this segment of the nucleolar-organizing body functions to produce a *large*, well-formed nucleolus, fig. 19. Furthermore, the segment from chromosome 9 possessed by chromosome 6^9 instead of being closely adjacent to the nucleolar-organizing body of chromosome 6^9, as in type-2 spores, fig. 9 and 18, is almost always *removed* some distance from the nucleolar organizing body. The possible significance of this difference will be brought out later, page 316.

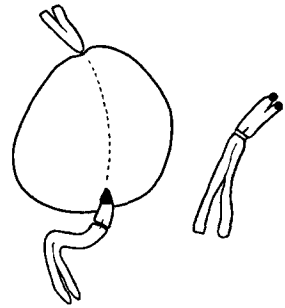

Fig. 19. The nucleolar association of chromosome 6^9 in a prophase of a type-3 spore. Chromosome 9 is to the right. Note, in this case, that the translocation segment on chromosome 6^9 is distantly removed from the nucleolar-organizing element.

The nucleolar condition in the nuclei of spore type-4 is unique. Type 4 spores possess a duplication for the satellite and a deficiency for two-thirds of the long arm of chromosome 9. The normal nucleolar-organizing body of chromosome 6 and the section of this body possessed by chromosome 9^6 are both present. However, these spores, instead of having one nucleolus with chromosome 6 and chromosome 9^6 attached, or two nucleoli with chromosome 6 attached to one and chromosome 9^6 to the other, showed many small nucleolar like bodies, photos. 24 and 41. Chromosomes 6 and 9^6, however, were usually associated with the largest of these bodies, fig. 20. Each of the other chromosomes had one or more of these nucleoli attached at indefinite positions along the chromosome. Detection of chromosomes 6 and 9^6 was made possible by means of their satellites and knobs. This was particularly clear in the type 4 spores of plants possessing the "large" satellite on chromosome 6 (page 307). In these spores, however, the satellites were usually only

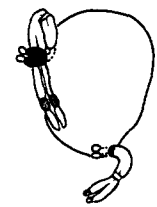

Fig. 20. Chromosome 6 and 9^6 in the prophase of a type-4 spore. Chromosome 6 has the "large" satellite. In this type of spore the satellites are not distantly removed from their respective nucleolar-organizing bodies.

a short distance removed from their nucleolar-organizing bodis. The presence of many nucleoli associated with any or every member of the chromosomal complement is in striking contrast to the situation found in spore types 1 and 3. A more detailed description of spore type 4 is given on page 317.

The telophase conditions resulting from the nuclear divisions within each of the four types of spores are essentially what would be expected from the observations of the prophases. In young anthers in which only some of the spores of types 1 and 2 have completed a nuclear division, there are but two classes of nuclei, those with one nucleolus and those with two nucleoli, one large and one small, as in photos. 18 and 19. Most of the latter class are undoubtedly related to spore type 2, the former class include spore type 1 and some of spore type 2 in which nucleolar fusions have occurred. In older anthers, in which the nuclei of spore types 1, 2 and 3 have undergone division and in which the nuclei of type-4 spores are undergoing division, there are three classes of spores with regard to nucleolar conditions: those with one nucleolus, those with two nucleoli, one large and one small, and those with many small nucleolar-like bodies, photos. 25 and 26.

The prophase and telophase observations indicate that spores with many small nucleoli belong mainly to type 4. The striking contrast between these spores with many small nucleoli and those with one or two well formed nucleoli, whether in the uninucleate or the binucleate condition, made it possible to determine their relative percentages. The results of such counts are given in table 3. The first three rows are counts

Table 3.

Culture	1 or 2 normal nucleoli	many nucleoli	Total	Percentage of spores with many nucleoli
13—14	305	100	405	24,7
13—14	624	214	838	25,5
13—22	313	95	408	23,3
13—17	434	146	580	25,2
13—22	688	164	852	19,2
13—35	377	114	491	23,2
46— 5	463	198	661	30,0
13—17	260	84	344	24,4
Totals	3464	1115	4579	24,4

from young anthers in which most of the spores where in prophase, only a few spores of types 1 and 2 having completed nuclear division. The remaining five rows represent counts from older anthers in which most of the spores were binucleated. The percentage of the different types of spores present in an anther depends entirely upon the relative percentages of the different types of distributions involving the four chromosomes of the interchange complex which occurred in the previous

anaphase. However, it can be seen from the total number that approximately 25 percent of the spores belong to the multinucleolated class, i. e., approximately 25 percent of the total number of spores are of type 4.[1]

The results of the counts of *A I* distributions suggested that in the main, four types of spores would be produced. The observations of the chromosome composition of the spores in the first nuclear division indicated that the anthers contained in the main, these four types of spores. The classifications and counts of the nucleolar conditions of the nuclei of the spores substantiate these observations and suggest that these four types of spores were present in approximately equal proportions.

The study of the nucleolar conditions in somatic tissue, in meiosis and in microspores of normal plants, plants homozygous for the interchange and plants heterozygous for the interchange, allows some definite conclusions to be drawn with regard to the development of the nucleolus. This will be considered in the following two sections.

Conclusions with regard to the development of the nucleolus.

The studies of this interchange have given rise to the following conclusions with regard to the development of the nucleolus. In nuclei which possess at least a full genome complement, the nucleolus is developed in the telophase through the functioning of the nucleolar-organizing element of chromosome 6 (the satellited chromosome). Haploid plants possess one satellited chromosome and thus one nucleolar-organizing element. The resting nuclei of these plants show only one nucleolus. In diploids, possessing two normal satellited chromosomes and thus two nucleolar-organizing elements, there are two nucleoli formed in the telophase. These may or may not fuse to form one nucleolus. In the microspore of diploid plants only a haploid complement is present. Only chromosome 6 possesses a nucleolar-organizing element. Here but one nucleolus is developed in each of the telophase nuclei resulting from the first nuclear division within the microspore. In plants trisomic for the satellited chromosome, there are three nucleoli developed in somatic telophase nuclei, fig. 3. Fusion of two or all three may occur. These three nucleoli are related to the nucleolar-organizing bodies of each of the three satellited chromosomes. Through meiotic distributions in these plants, spores with ten or eleven chromosomes are produced. Those with ten chromosomes have the normal monoploid complement with one

[1] The counts can be considered only as a close approximation and not as an exact indication of the number of type 4 spores present. Spore types 9 to 14 can resemble spore type 4 in nucleolar condition. However, the percentage of such spores is relatively low and should not interfer with the conclusions that approximately 25 percent of the spores are of type 4.

satellited chromosome and thus but one nucleolar-organizing element. Those with eleven chromosomes have two satellited chromosomes and thus two nucleolar-organizing elements. The telophase nuclei resulting from the first nuclear division within the spore are characterized by the presence of one or two nucleoli. Those spores with two nucleoli are undoubtedly eleven-chromosome carrying spores. Futhermore, there is no characteristic size difference between the two nucleoli in these nuclei. The nucleoli are usually approximately similar in size, photo. 21.

The interchange described in the previous pages divided the nucleolar-organizing body into two unequal parts, each of the two interchanged chromosomes possessing a section of it. Although the total amount of nucleolar-organizing substance is theoretically the same in both diploids and plants homozygous for the interchange the former are characterized by the development of two nucleoli, usually of similar size, the latter by the development of four nucleoli, two large and two small, fig. 5. The monoploid spores produced by plants homozygous for the interchange have one each of the two interchanged chromosomes. The telophase nuclei resulting from the first nucleolar division within these spores are characterized by the production of two nucleoli, one large and one small, in corresponding positions within the two sister nuclei, photos. 18, 29 and 20.

Plants which are heterozygous for the interchange possess one normal satellited chromosome with a complete nucleolar-organizing body and the two interchanged chromosomes each with a section of the nucleolar-organizing body. In the somatic telophases, three nucleoli are developed, two large and one small, Fig. 4. The two large nucleoli are developed by the functioning of the nucleolar-organizing element of the normal chromosome 6 (satellited chromosome) and the small segment of the nucleolar-organizing body of the interchanged chromosome 9^6, fig. 2, whereas, the small nucleolus is developed by the larger segment of the nucléolar-organizing body carried by the interchanged chromosome 6^9. Evidence for the development of the smaller nucleolus by the segment of the nucleolar-organizing body carried by chromosme 6^9 has been given in the previous pages. It can be reviewed briefly. (1) In the meiotic prophases in plants homozygous for the interchange, two nucleoli, one large and one small, are sometimes present. In these cases, chromosome bivalent 9^6 is always associated with the larger nucleolus, chromosome bivalent 6^9 with the smaller nucleolus. (2) In the prophase of the first nuclear division within the microspore similarily, two nucleoli are sometimes present. In these cases, also chromosomes 9^6 is always associated with the larger nucleolus, chromosome 6^9 with the smaller nucleolus. (3) In the meiotic prophase of plants heterozygous for the interchange very occasionally two distinct nucleoli are present, associated

with the chromosomes involved in the interchange. Here again, there is a distinct size difference with chromosomes 6 and 9^6 associated with the larger nucleolus and chromosome 6^9 with the smaller nucleolus, fig. 14, photo. 7. (4) A definite size difference, so characteristic for nucleoli of plants homozygous or heterozygous for the interchange, is not found in plants possessing three normal satellited chromosomes, either in the somatic telophase nuclei or in the microspore telophase nuclei. With few exceptions[1], the nucleoli formed are not strikingly different in size. The small segment of the nucleolar-organizing body possessed by chromosome 9^6 is that region of the nucleolar-organizing body which in the normal chromosome 6 lies immediately adjacent to the nucleolus. Chromosome 6^9 retained the larger segment. This region of the deep-staining nucleolar-organizing body in the normal chromosome 6 is frequently free from direct contact with the nucleolus. Taken as a whole, the evidence suggests that in the normal satellited chromosome the distal region of the nucleolar-organizing body (that which is farthest from the spindle fiber attachment region) is mainly responsible for the orderly organization of the nucleolus. In other words, the different regions of the nucleolar-organizing body of the normal satellited chromosome possess differential capacities for developing nucleolar substance.

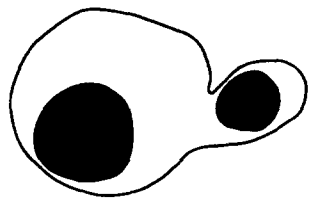

Fig. 21. See footnote page 316.

As has been described on page 309, plants heterozygous for the interchange produced four main types of microspores. Type-1 spores have the normal monoploid complement with one satellited chromosome and one nucleolar-organizing body. These spores always show one nucleolus. Type-2 spores, with the interchanged complement, possess segments of the nucleolar-organizing body on two chromosomes. These spores have either one nucleolus with chromosomes 6^9 and 9^6 attached to it or two nucleoli with chromosome 9^6 attached to the larger and chromosome 6^9 to the smaller nucleolus. Type-3 spores have a normal chromosome 9 and 6^9. These are deficient for the satellite of chromosome 6 and have a duplication for two thirds of the long arm of chromosome 9. In these spores only a segment of the nucleolar forming element, that possessed by chromosome 6^9 is present. It should be emphasized that in these spores there is one *large* nucleolus present. Thus, the capacity of this segment of the nucleolar-organizing body to develop a normal sized nucleolus is definitely proven. This evidence indicates that when nucleolar-organizing elements are present in several chromosomes of a complement, the size of the nucleolus developed by each will be

[1] See page 316.

determined, in the main[1], by its particular capacity for functional activity. If the nucleolar-organizing elements have similar functional capacities, nucleoli of similar size will be formed. If, however, there is a differential rate of functioning the nucleolar-organizing element with the greater rate of functional capacity will develop the larger nucleolus, that with the slower rate of functional capacity will develop a smaller nucleolus. When a nucleolar-organizing element with a slower rate of functional capacity is present alone, it develops just as large a nucleolus as a nucleolar-organizing element with a more rapid rate of functioning.

This evidence may help to interpret the peculiar results NAWASHIN (1927) obtained in a species cross between *Crepis capillaris* × *Crepis tectorum, Crepis capillaris* × *Crepis parviflora, Crepis foetida* × *Crepis rubra*. Each species possesses a satellited chromosome in the monoploid complement which is readily recognizable in the metaphase figures. In each of the crosses enumerated above, only one satellited chromosome was found in the metaphase figures in the resulting individuals. This satellited chromosome could be assigned to a particular parent. The satellited chromosome of the other parent was present but no stalk separating the satellite from the main body of the chromosome was present. Instead, the satellite was directly adjacent to the main body of the chromosome. Since it has been stated on page 298 that the presence of a visible satellite in metaphase is dependent upon the production of a nucleolus by this chromosome in the previous telophase, the absence of a satellite at metaphase suggests that this chromosome did not function to produce a nucleolus or produced a very small one which did not remove the satellite from the main body of the chromosome. Very occasionally in spores of plants homozygous for the interchange, the nucleolar-organizing body of chromosome 6[9] fails entirely to function. This chromosome then lies free in the nucleus, showing no relationship with the nucleolus and no indication of an achromatic stalk between the visible nucleolar-organizing body and the interchanged segment. If, in these species crosses, the rate of functioning of the nucleolar-organizing element of the satellited chromosome of one species is far greater than that of the other species, competitive activity may result in the total disability of the satellite chromosome from one species to produce a nucleolus and thus a complete failure of the satellite to become drawn away from its

[1] A second factor governing size of nucleoli can be detected when two chromosomes with nucleolar-organizing elements of similar rates of functional capacity are present in a nucleus. Normally, in a telophase nucleus, these two chromosomes would develop approximately similar-sized nucleoli. If, however, one of these chromosomes is at the outer edge of an anaphase figure and enters the telophase state as a small pocket attached to the main part of the nucleus by a narrow isthmus, the nucleolus developed by this chromosome is much smaller than the nucleolus produced by the other chromosome in the main part of the nucleus, photo. 23, fig. 21. It is as if it had less substance available (matrix, see page 318) with which to organize a nucleolus.

nucleolar-organizing element through nucleolar enlargement. Should this be true, the functional capacity of the nucleolar-organizing element of this chromosome should reappear when backcrossed to its parental form. The satellite on this chromosome would again be visible[1].

The nucleolar situation in the type-4 spore produced by plants heterozygous for the interchange opens up an entirely different aspect to the production and development of nucleoli. Here, there are two nucleolar-organizing elements present, one on chromosome 6 and one on chromosome 9[6]. However, instead of one or two nucleoli being present, there are often many small nucleoli each associated in some way with a chromosome of the complement. It is obvious, therefore, that nucleolar formation in nuclei with particular genomic deficiencies can be independent of the nucleolar-organizing elements. This seems contractory to the above observations and statements. HEITZ, 1931 b, noticed this same contradiction. He reports that in *Vicia faba* and *Vicia monanthus,* which have only two SAT-chromosome (see page 295) in the diploid complement, two cases were found where there was present besides the two main sister nuclei, a small extra nucleus. The small nucleus contained a distinct though small nucleolus. The small nucleus probably arose from a chromosome which lagged during anaphase and thus was excluded from the organizing sister nuclei. Since each of the main nuclei possessed two nucleoli, HEITZ concluded that they contained the two SAT-chromosomes. Therefore, the small nucleus was derived from another chromosome of the complement, not a SAT-chromosome. However, it had a nucleolus. This caused him to revise his earlier (1931 a) statement to read as follows: „Immer wenn SAT-Chromosomen vorhanden sind, *müssen* an ihnen Nukleolen entstehen. Die SAT-Chromosomen wirken begrenzend auf die Zahl, Lage und Größe der Nukleolen. Fehlen die SAT-Chromosomen, so können sich die Nukleolen trotzdem bilden", page 504, 1931 b. The type-4 spore formed by plants heterozygous for the interchange, which possess two "SAT-chromosomes" but show many "nucleoli" associated with other chromosomes of the complement, indicate that the first sentence in the quotation can be modified. The mere presence alone of the "SAT-chromosome", with its nucleolar-organizing element, does not insure that an orderly organization of the nucleolus will occur. Under certain conditions and with certain genomic deficiencies, the nucleolar-organizing elements can not function properly.

A possible explanation of this discrepancy is strikingly suggested by the behavior of the matrix substance of the chromosome. This will be described in the following section.

Possible explanation of the nucleolar condition of type-4 spores.

The increased stainability of chromosomes with the disappearance of the nucleolus in the late prophase is a matter of common observation.

[1] See addendum (p. 326).

The evidence obtained in this study suggests that: (1) the nucleolus substance contributes to the matrix of the chromosome (a similar suggestion has been made by MARSHAK, 1931, and DERMEN, 1933) (2) that this substance enters the chromosome at the late prophase, and contributes to the visible matrix, (3) and that after a considerable swelling of this matrix material in the late anaphase and early telophase, a definite nucleolus is reorganized from its substance under the influence of the nucleolar-organizing element of the satellited chromosome. Should, however, such a nucleolar-organizing element be absent or fail to function, the expanded matrix material of each chromosome remains with the individual chromosome, collecting at different regions along the chromosome into nucleolar-like droplets. The following evidence is presented which supports this contention.

In the mid-anaphase of the first meiotic mitosis the matrix substance of the chromosome swells and is visible as a light-staining rim completely surrounding the arms of the chromosome. The deep-staining chromatin is thus embedded in a light-staining matrix sac, photo. 42. Similar appearances in animal material have been observed by a number of workers, see RICHARDS, 1917. As the chromosomes approach the poles, the matrix substance of the chromosomes sends out processes which anastomose with one another. By this time, the chromatin has begun to disperse within the matrix sac. This dispersion of the chromatin within the anastomosing matrix substance gives the appearance of actual anastomosing of the chromatin. This, I believe, is not necessarily true. It is the matrix substance which anastomoses, carrying with it the dispersing chromatin. This anastomosing of matrix substance proceeds further until all chromosomes are in contact by means of their matricies, photo. 43. The individual chromosomes are still visible although their chromatin is much dispersed. About this time, in many cells, two small nucleoli appear within the matrix substance. Their position suggests that they have been developed by the two nucleolar-organizing elements of the two diverging arms of the X-shaped satellited chromosome. Fusion frequently occurs between these two nucleoli. However, the nucleoli thus formed usually do not develop into well rounded nucleoli such as seen in somatic nuclei. Instead, they frequently remain irregular in shape and definitely confluent with the fused matrix substance of the chromosome. In other parts of the nucleus small nucleolar-like bodies are frequently visible. The following prophase indicates that these are accumulations of the expanded matrix substance. In the earliest prophase, the long X-shaped chromosomes (dyads held together at the spindle fiber attachment region) are readily visible. Each is embedded in a lightly staining matrix which completely surrounds the four arms of the X-shaped chromosome, photo. 44. The matrices of several of the chromosomes usually are confluent with the true nucleolus and with

the small nucleolar-like bodies. As the prophase proceeds, the chromatin forming the arms of the X-shaped dyads contracts and becomes more stainable. Reduction in the amount of visible matrix substance is correlated with the contraction and the increased stainability of the chromosomes. In late prophase, the lightly staining matrix substance is decidedly reduced in amount and appears as a thin rim about each chromosome, photos. 45 and 47. At this stage, it can be determined that what has been called the true nucleolus is associated with the chromosome possessing a nucleolar-organizing body. This nucleolus, however, is still conjoined with the matrices of several chromosomes, photo. 45. As the chromosomes contract still further and become more deeply stained, very little matrix substance is visible about the arms of the chromosomes, photo. 46. By this time, the definitive nucleolus is much reduced in size and becomes released from the nucleolar-organizing body and the matrices of the chromosomes. This stage is very late prophase, Metaphase *II* shows short, deep-staining chromosomes. There is usually no nucleolar remnant visible.

The following anaphase (anaphase *II*) repeats in all essential details the situation described for anaphase *I*. At late anaphase the matrix begins to swell about each chromosome. Anastomoses commence between the matrices of the different chromosomes and the chromatin begins to appear more diffuse. By continued coalitions of matrices, the nuclear membrane is formed. The nucleolus develops in the midst of the fused matrix substance. Thus a number of chromosome matrices are definitely joined with the forming nucleolus. Frequently, however, in these young spores nucleolar-like bodies, believed to be accumulations of the expanded matrix substance and not true nucleoli, are observed distributed in the nucleus. Gradually, as the spore matures, these nucleolar-like bodies disappear. By the time that the first nuclear division in the microspore occurs, there is but one nucleolus, the smaller nucleolar-like bodies having disappeared. Occasionally, however, a premature division occurs in one or two spores of an anther. If this occurs before the small nucleolar-like bodies have disappeared, they can be seen in the late prophase attached to the several chromosomes of the complement. Such a premature prophase is illustrated in photo. 38. Note the main nucleolus associated with the satellited chromosome in the region of the nucleolar-organizing body and the smaller nucleolar-like bodies associated with the other chromosomes.

The process in somatic anaphase and telophase is similar to that described for *I* and *II*. Here, however, the rate of disappearance of the matrix and the formation of the definitive nucleolus is much faster. The nucleolus developes rapidly in very early telophase. Here, likewise, it first appears in the fused substance. Thus, many chromosome-matrices appear to be joined to the nucleolus at this stage. The nucleolus, however,

develops at the position of the nucleolar-organizing body. This can be determined in the early telophases of those somatic nuclei in which the chromosomes are still identifiable. In somatic telophases, the extra nucleolar-like bodies observed in *T I* and *T II* are usually not found. This may be correlated with the more rapid rate of development of the nucleolus in somatic tissue and consequently, the more rapid removal of the matrix substance.

As has been stated, type-4 spores showed many nucleolar-like bodies in the resting and prophase stages. Usually, however, there were one or two larger nucleoli, photo. 40. Observation of the late prophases indicate that the largest nucleolus is associated with chromosomes 6 and 9^6, fig. 20. The relation of the small "nucleoli" to the other chromosomes becomes visible as the nuclei proceed into the late prophase stage, photos. 24 and 41. These "nucleoli" are definitely associated with the chromosomes, one or more at different positions along the chromosome. In some cases, it appears as if the whole chromosome were embedded in the matrix (nucleolar) substance which, however, has mostly accumulated at one or two positions to form these droplets of nucleolar-like substance. This nucleolar-like substance of one chromosome is frequently fused with that of another. As the spores approach metaphase, the number and size of the nucleolar-like bodies decreases and the chromosomes become more stainable. There are frequently, however, severall small "nucleoli" visible at metaphase. The telophases of these spores are characterized by the production of many nucleoli-like bodies, photo. 25, in contrast to the normal spores which have one (spore types 1 and 3) or two (spore type 2) nucleoli, photos. 18, 19 and 20. Compare, also, photo. 26, the generative nucleus of a type-4 spore with photo. 27, the generative nucleus of a type-1 spore.

Spore quartets were examined in plants heterozygous for the interchange to see if a segregation for this type of spore could be determined. In most cases, it was not possible to classify the spores of a quartet with regard to nucleolar condition because of the presence of the many nucleolar-like bodies (mentioned above) in addition to the small true nucleolus or nucleoli of spore types 1 to 3. At this stage, therefore, a distinction between type-4 spores and those of types 1 and 3 could not be clearly seen. As has been stated, page 319, these nucleolar-like bodies disappear in spore types 1, 2 and 3 during the maturing of the spore. It is probable that in spore type 4 they do not disappear but remain and possible increase in size as the spores matures. However, there were a few spore quartets with a definite segregation: two spores with normal nucleoli and two with many small nucleolar-like bodies and no definitive nucleolus, photo. 36. The lower left spore is shown with greater magnification in photo. 48. Such spore quartets are believed to be produced as the result of 3—1 distributions of the four chromosomes in the first

meiotic anaphase with chromosomes 6, 6^9, 9^6 going to one pole and chromosome 9 going to the opposite pole. Evidence for such a distribution has been given in the footnote on page 310.

That a chromosome complement deficient for the region of chromosome 6 which includes the nucleolar-organizing body will produce a nucleus with many small nucleolar-like bodies, as in photos. 36 and 48, has been established by the investigations of Miss CREIGHTON (unpublished). In a plant resulting from the functioning of X-rayed pollen one chromosome 6 was found to be deficient for most of the short arm. The other chromosome 6, contributed by the untreated female parent, was normal and possessed a nucleolar-organizing body. In somatic telophase nuclei of this plant only *one nucleolus* developed. This is correlated with the fact that only one nucleolar-organizing element is present in the somatic chromosome complement. The distribution of these two chromosomes in meiosis should produce spores, one half of which carry a normal satellited chromosome with a nucleolar-organizing element and one-half with the deficient chromosome and no nucleolar-organizing element. Two strikingly different types of spores, present in equal proportions, were found in the anthers of this plant. The spores with the normal chromosome 6 possessed one well developed nucleolus, as in photo. 37. Spores with the deficient chromosome 6 had many small nucleoli, as in photo. 39. Examination of the prophases of the first nuclear division in the microspore revealed that in these latter spores, the relation of the small nucleolarlike bodies to the chromosomes were essentially similar to that described for type-4 spores, compare photos. 24 and 41.

From this case alone it could be concluded that if a normal nucleolus is not developed through the functioning of a nucleolar-organizing element, nucleolar substance present in each chromosome or produced by each chromosome, will accumulate in nucleolar-like droplets along the chromosome. In other word, the function of the nucleolar-organizing element of chromosome 6 apparently is to organize the nucleolar substance present in each chromosome into a definite body, the nucleolus. With regard to its nucleolar condition, type-4 spore can be placed mid-way between spore types 1 to 3, with their well developed nucleoli and no small nucleolar-like bodies, and the spore type described above (having no nucleolar-organizing body) with no definite nucleolus and many small nucleolar-like bodies. It possesses a small true nucleolus associated with the nucleolar-organizing bodies of chromosomes 6 and 9^6 and in addition, many small nucleolar-like bodies associated with the other chromosomes of the complement. It is probable that the deficiency present in this chromosome complement hinders the metabolic activity to such an extent that the nucleolar-organizing elements of chromosomes 6 and 9^6 are unable to function fully and therefore to remove the nucleolar substance from each chromosome in the organization of a true nucleolus. In

consequence, this substance in each chromosome accumulates into nucleolar-like droplets.

That the material the nucleolar-organizing element uses to organize a nucleolus is the so-called matrix substance of the chromosome is difficult to state with certainty. However, from the observations described in this section it is also difficult to avoid the impression that a distinct relationship exists between the two. The behavior of the matrix substance (nucleolar substance?) in the anaphase and telophase (page 319) suggests that it may be concerned with the distribution and dispersion of the chromatin within a chromosome into the metabolic condition. It is possible that complete release of the matrix from the chromatin is necessary before the chromatin can function properly in metabolism.

Summary.

1. The nucleolus is organized in the telophase through the activity of a distinct deep-staining body having a definite position in one chromosome (the satellited chromosome) of the monoploid complement. Correlated with the number of satellited chromosomes present, the telophases of somatic tissue of haploids show one nucleolus, diploids, two nucleoli and triploids, three nucleoli. That the nucleolus develops through the activity of this body (refered to as the nucleolar-organizing body or element) was obtained from a reciprocal translocation which broke this body into two parts. Both interchanged chromosomes possessed a section. Nucleoli developed from *each* of these two segments. Thus, plants homozygous for the interchange developed four nucleoli in their somatic telophases; plants heterozygous for the interchange developed three nucleoli in their somatic telophases. Similarly, the telophase nucleoli resulting from the first division within the monoploid microspore of normal diploids show only one nucleolus, whereas, those of plants homozygous for the interchange are characterized by the development of two nucleoli.

2. The functional capacity to develop a nucleolus is not the same for both segments of the severed nucleolar-organizing body. This is evident when the two interchanged chromosomes are present in the same nucleus. The segment of the nucleolar-organizing body possessed by one interchanged chromosome produced a large nucleolus, whereas, the segment of the nucleolar-organizing body possessed by the other interchanged chromosome produced a small nucleolus. When this latter chromosome, with the nucleolar-organizing element of slower rate of functional capacity is present without the former (i. e. without a competing nucleolar-organizing element) it produces, in contrast, a large nucleolus.

3. The activity of the nucleolar-organizing element is hindered by certain genomic deficiencies. When this occurs, many small nucleolar-like bodies are produced and remain associated with the other chromosomes of the complement. These small nucleoli appear to develop from

a swelling and later collection into droplets of the matrix substance of the chromosome.

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Photographs.

Plate VIII.

Magnifications of photos. 1 and 2: × 1300.

Photo. 1. Two photographs of the same cell taken at different optical planes. See diagram of same, fig. 6. a, note nucleolar-organizing body of chromosome 6^9. c, shows chromosome 9^6.

Plate 2: Three photographs of the same cell taken at different optical planes. See sketch of same, fig. 7. a, shows nucleolar-organizing bodies of chromosomes 9^6 and 6^9, translocated segment on chromosome 6^9 and short arm of chromosome 9^6 c, shows the satellite of chromosome 9^6 and the spindle fiber attachment region of this chromosome.

Photo. 3: See sketch of same, fig. 8. Magnification × 880.

Plate IX.

Magnifications of photo. 4, × 880; photos. 5 to 9, × 1300; photo. 10, × 1200.

Photo. 4: Same type of configuration shown in photo. 3. Bivalent chromosome 9^6 is associated with the larger nucleolus; bivalent chromosome 6^9 is associated with the smaller nucleolus.

Photo. 5: See sketch of same, fig. 12.

Photo. 6: See sketch of same, fig. 13.

Photo. 7: See sketch of same, fig. 14.

Photo. 8: See sketch of same, fig. 15.

Photo. 9: Early diplotene in plant heterozygous for the interchange. The chromosomes forming the cross-shaped configuration have commenced to "open-out". Chromosomes 6, 9^6 and 6^9 are associated with the nucleolus, whereas, chromosome 9 is free from the nucleolus. Note that the terminal knot (upper right) is heterozygous. Chromosome 9 possessed a terminal knob, chromosome 9^6 was knobless.

Photo. 10. The homozygous association of the nucleolar-organizing bodies (arrow) of chromosome 6. The associated satellites lie above. Most of the nucleolus and the rest of the chromosome 6 bivalent were not in the photographed plane

Plate X.

Magnifications of all photos: × 1300.

Photo. 11. Diakinesis configuration in a plant heterozygous for the interchange. The order of the chromosomes from left to right: 6, 6^9, 9, and 9^6. Note the satellite of chromosome 6.

Photo. 12: Diakinesis ring configuration in a plant heterozygous for the interchange. The small nucleolus is attached to the nucleolar-organizing element of chromosome 6^9.

Photo. 13: Metaphase ring configuration (arrow) showing "alternate" distribution of the chromosomes.

Photo. 14: Metaphase chain configuration showing satellites at free ends of the chain. a, normal satellite (arrow) at free end of chromosome 9^6. b, "large" satellite (arrow) at free and of chromosome 6.

Photo. 15: Metaphase ring configuration (center of photograph) showing "adjacent" distribution of chromosomes.

Photo. 16: Metaphase chain configuration (extreme left) showing "alternate" distribution of chromosomes.

Photo. 17: Metaphase chain configuration (extreme left) showing "adjacent" distribution of chromosomes.

Plate XI.

Magnifications of all photos × 720.

All photographs are of the late telophase of the first nuclear division in the microspore.

Photo. 18: Telophase of microspore from plant homozygous for the interchange. Each nucleolus has one chromosome 6^9 and one chromosome 9^6. Note the large and small nucleolus in each sister nucleus.

Photo. 19: Same situation as shown in photo. 18.

Photo. 20: Same situation shown in photos. 18 and 19 except that the two nucleoli in the generative nucleus (upper) have commenced to fuse.

Photo. 21: Telophase nuclei of a microspore from a plant trisomic for chromosome 6. The two sister nuclei each contain two nucleoli of approximately similar size. This is undoubtedly an 11-chromosome spore, the two nucleoli being produced by the functioning of the nucleolar-organizing bodies of each chromosome 6.

Photo. 22: Telophase nuclei in a microspore from a plant heterozygous for the interchange. Each nucleus has three nucleoli, two large and one small. The nucleoli in the generative nucleus (left) have commenced to fuse. This is probably an 11-chromosome spore with chromosomes 6, 9^6, and 6^9. Chromosomes 6 and 9^6 produced the large nucleoli, chromosome 6^9, the small nucleolus.

Photo. 23: See sketch of same, fig. 21.

Plate XII.

Magnifications of photos. 24 and 25: × 720, photos. 26 to 35: × 1300.

Photo. 24: Late prophase of first nuclear division of type-4 spore.

Photo. 25: Telophase of first nuclear division of type-4 spore.

Photo. 26: Generative nucleus in a type-4 spore. Note the many small nucleolar-like bodies. Compare with normal generative nucleus, photo. 27.

Photo. 27: Generative nucleus from a normal microspore. There is one large nucleolus.

Photo. 28: Prophase of the first nuclear division in a plant homozygous for the interchange. See sketch of same, fig. 9.

Photo. 29: Prophase of the first nuclear division in a plant homozygous for the interchange. There are two nucleoli, one large and one small (arrow). Chromosome 6^9 is associated with the small nucleolus, chromosome 9^6 with the larger nucleolus.

Photo. 30: Metaphase of the first nuclear division in a normal microspore. The arrow points to chromosome 6 with its satellite.

Photo. 31: Metaphase of the first nuclear division in a microspore from a plant homozygous for the interchange. See sketch of same, fig. 11.

Photo. 32: Chromosome 6 with the "large" satellite (arrow) in prophase of the first nuclear division of a microspore.

Photo. 33: The "large" satellite of chromosome 6 in prophase of a microspore.

Photo. 34: Late prophase of the first nuclear division in an 11-chromosome microspore in which chromosome 6 with a "large" satellite and chromosome 9[6] with a normal satellite are present. See sketch of same, Fig. 16. a, shows the small "normal" satellite, b, the "large" satellite.

Photo. 35: The "large" satellite of chromosome 6 in the prophase of the first nuclear division of a microspore.

Plate XIII.

Magnification of photo. 36: × 720, photo. 37 to 41: × 1300.

Photo. 36: Spore quartet from a plant heterozygous for the interchange. The two spores to the left have many small nucleolar-like bodies, the two spores to the right each have one large and one small nucleolus. See lower left spore at greater magnification, photo. 48. For explanation see page 321.

Photo. 37: Young microspore with one large well organized nucleus.

Photo. 38: Premature first nuclear division in a microspore. For explanation, see page 319.

Photo. 39: Young microspore with many small nucleolar-like bodies and normal nucleolus. The nuclear membrane has broken in the flattening of the cell. Compare with normal condition, photo. 37.

Photo. 40: Nucleus of a type-4 spore shortly before prophase of the first nuclear division. Note that there is one medium sized nucleolus and many small nucleolar-like bodies.

Photo. 41: Late prophase of the first nuclear division of a type-4 spore. Note the many nucleolar-like bodies associated with the chromosomes.

Plate XIV.

Magnifications: × 1300.

Photo. 42: Anaphase *I*. Two groups of chromosomes in upper part of figuer show the commencement of the fusion of the matrix substance.

Photo. 43: Telophase *I*. Later stage in the fusion and anastomoses of the matrix substance of the chromosomes.

Photo. 44: Early prophase *II*. The arms of the X-shaped chromosomes are embedded in matrix. The matrices of several chromosomes are fused.

Photo. 45: Late stage of prophase II. The chromosomes are shorter and more deeply stained. The matrix substance about the arms of the chromosomes is decreasing.

Photo. 46: Very late prophase *II*. A thin rim of matrix substance is still visible about each chromosome.

Photo. 47: Late prophase *II*. Note the matrix about the X-shaped chromosomes.

Photo. 48: Lower left spore from quartet shown in photo. 36. Note the small nucleolar-like bodies.

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Addendum.

Shortly after sending this paper to press my attention was called to the recent publication of NAWASHIN (Chromosome alterations caused by hybridization and their bearing upon certain general genetic problems) [1], in which more information is given concerning the "disappearance" of the satellite of one parental chromosomal complement in the F_1 of *Crepis* species crosses. The hypothesis stated on page 316 of this paper

[1] Cytologia **5**, 169—203 (1934).

to account for the "disappearance" of the satellite finds substantiation in the new results recorded by NAWASHIN. In this hypothesis, it is assumed that the rate of functional capacity of the nucleolar-organizing elements of the satellited chromosomes of different species of *Crepis* varies. This differential rate of activity of the nucleolar forming elements of different satellited chromosomes will exhibit itself only when two such chromosomes are present in the same nucleus. The chromosome having a nucleolar-organizing element with the grater speed of reaction will utilize the available material for production of a nucleolus in advance so that the chromosome with the nucleolar-organizing element of slower rate of functioning will have little or no opportunity to function. Therefore, in the latter chromosome, with little or no production of a nucleolus, the satellite cannot be removed from the nucleolar-organizing element through growth of a nucleolus. Consequently, the satellite remains adjacent to the nucleolar-organizing element and no satellite stalk is produced. Through appropriate crosses it is possible to determine the relative rates of functional activity of the nucleolar-organizing elements of the different *Crepis* species. The tabulated results given by NAWASHIN allow a beginning of such a gradation to be made.

In the following table (Table 4) taken from page 174 of NAWASHINs publication, no disappearance of the satellite of either parent was noticed indicating that the relative rates of functioning of the nucleolar-organizing elements in the two species crossed are not vastly different. It is expected, however, that observations of the size of the nucleolus formed by each parental satellited chromosome in these hybrids will give an indication of differences in the rates of nucleolar forming activities of their respective parental satellited chromosomes.

Table 4.

C. alpina × *C. dioscoridis*
C. capillaris × *C. aspera* (M. NAWASHIN, 1927, 1928)
C. „ × *C. aculeata*
C. „ × *C. setosa*
C. leontodontoides × *C. aurea* (P. AVERY, 1930)
C. rubra × *C. foetida* (C. POOLE, 1930)
C. tectorum × *C. alpina* (M. NAWASHIN, 1928)
C. „ × *C. neglecta*

The following table (Table 5) from NAWASHIN, summarizes the information with regard to the "disappearance" of the satellite in additional species crosses.

From this table it can be concluded that the rate of functional activity of the nucleolar-organizing element of *C. capillaris* is greater than that of *alpina, dioscoridis, neglecta, tectorum* and *leontodontoides*. However, the functional activity of the *parviflora* nucleolar-organizing element is greater than that of *capillaris*. Therefore, when *parviflora* is crossed

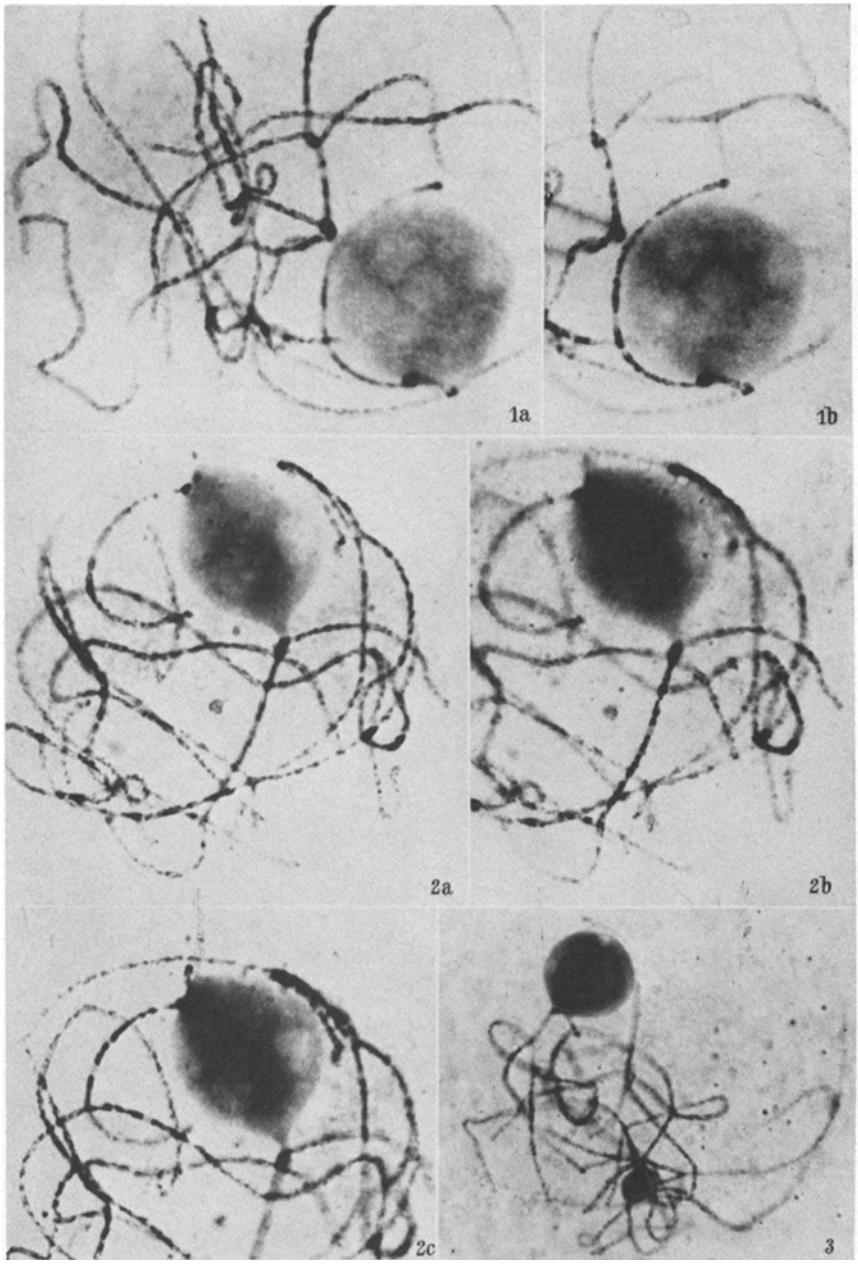
Table 5.

Combination	The satellite "disappears" from the chromosome of	Remarks
C. capillaris × *C. alpina*	*C. alpina*	M. NAWASHIN, 1928
C. ,, × *C. dioscoridis*	*C. dioscoridis*	,, 1928
C. ,, × *C. neglecta*	*C. neglecta*	,, 1928
C. ,, × *C. parviflora*	*C. capillaris*	,, 1927
C. ,, × *C. tectorum*	*C. tectorum*	,, 1927
C. dioscoridis × *C. pulchra*	*C. pulchra*	,, 1928
C. leontodontoides × *C. capillaris*	*C. leontodontoides*	P. AVERY, 1930
C. ,, × *C. marschallii*	*C.* ,,	,, 1930
C. ,, × *C. parviflora*	*C.* ,,	,, 1930
C. ,, × *C. tectorum*	*C.* ,,	,, 1930
C. palestina × *C. pulchra*	*C. pulchra*	M. NAWASHIN, 1934
C. parviflora × *C. tectorum*	*C. tectorum*	,, 1928
C. setosa × *C. tectorum*	*C.* ,,	,, 1928

to *alpina, dioscoridis, neglecta, tectorum* and *leontodontoides* the disappearance of the satellite of these latter species should result in the hybrids. In the table the results of two of these crosses, that of *tectorum* and *leontodontoides*, are given. In these two cases, the hypothesis is substantiated, the satellites of *tectorum* and *leontodontoides* have "disappeared".

Since, as table 4 shows, there is not a striking difference in the functional activities of the nucleolar-organizing elements of the satellited chromosomes of *capillaris* and *setosa* the presence of a *setosa* chromosomal set in the hybrids with *alpina, dioscoridis, neglecta, tectorum* and *leontodontoides* should result in the "disappearance" of the satellites of the latter species in these hybrids. Only one of these crosses, that of *setosa* × *tectorum* is given in table 5, and, as expected it is the *tectorum* satellite which "disappears". By similar reasoning through extended investigations it should be possible to produce a seriated arrangement of *Crepis* species with regard to funtional capacities of the nucleolar-organizing elements of the satellited chromosome of each species.

As would be expected on this, the satellite which "disappeared" should reappear in those F_2, F_3 etc. plants which habe only this particular type of satellited chromosome present in their nuclei. In the two cases that NAWASHIN investigated, this has been confirmed.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1b
2a
2b
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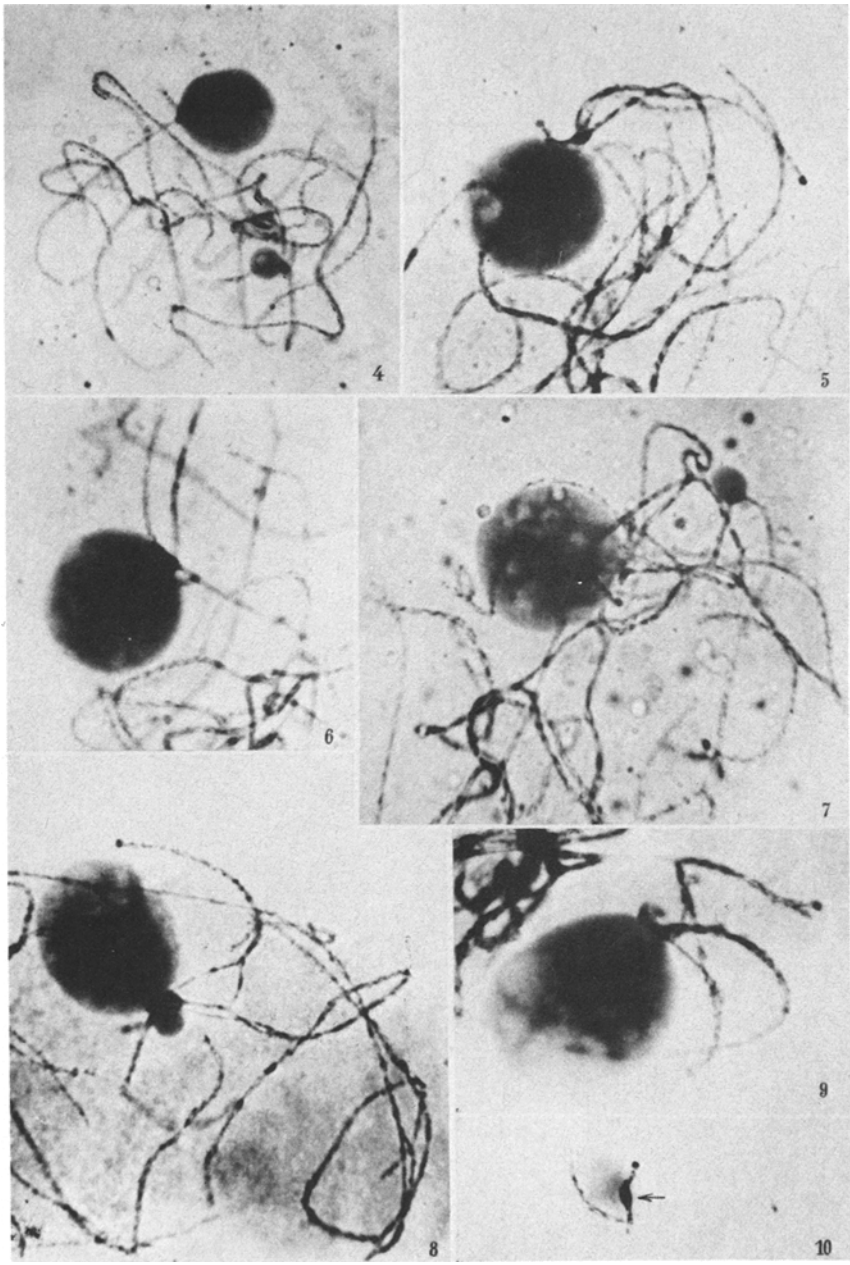
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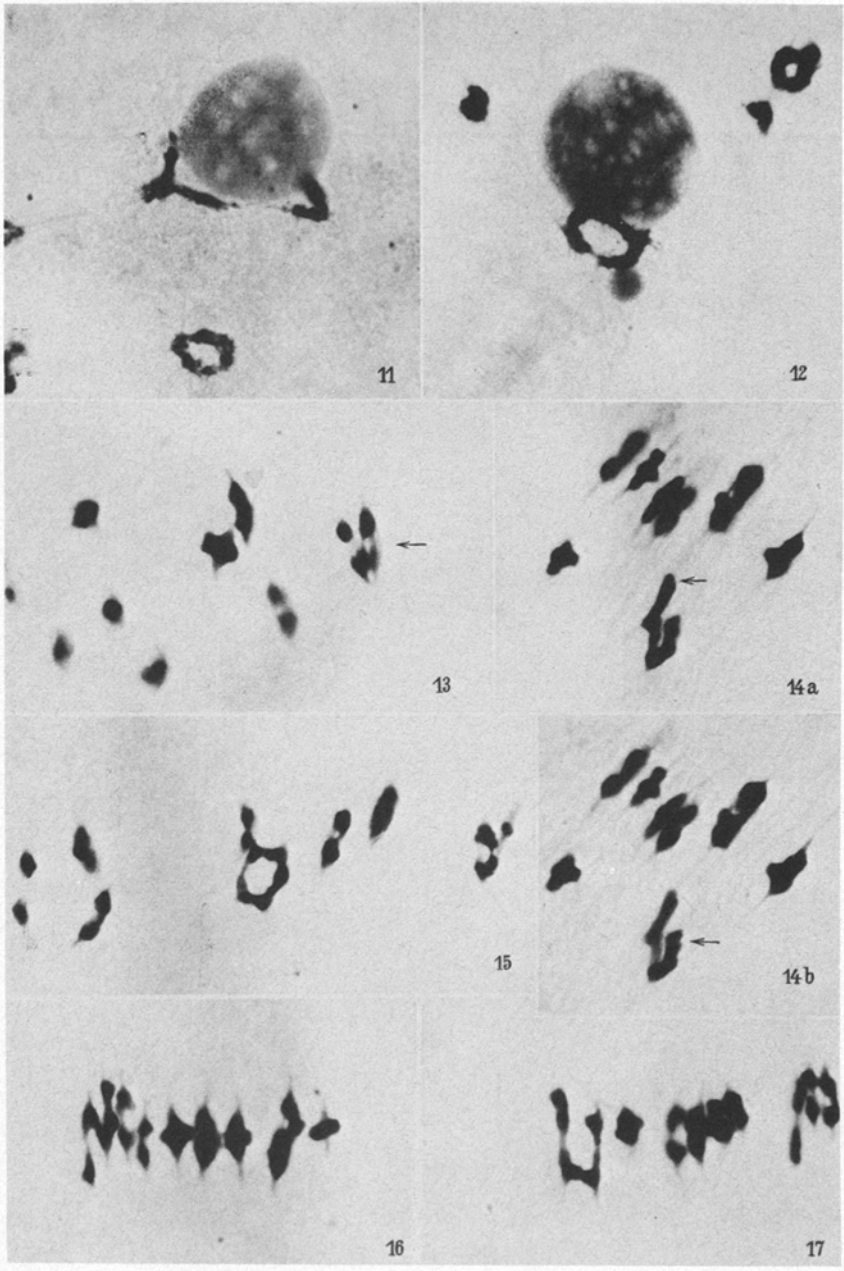
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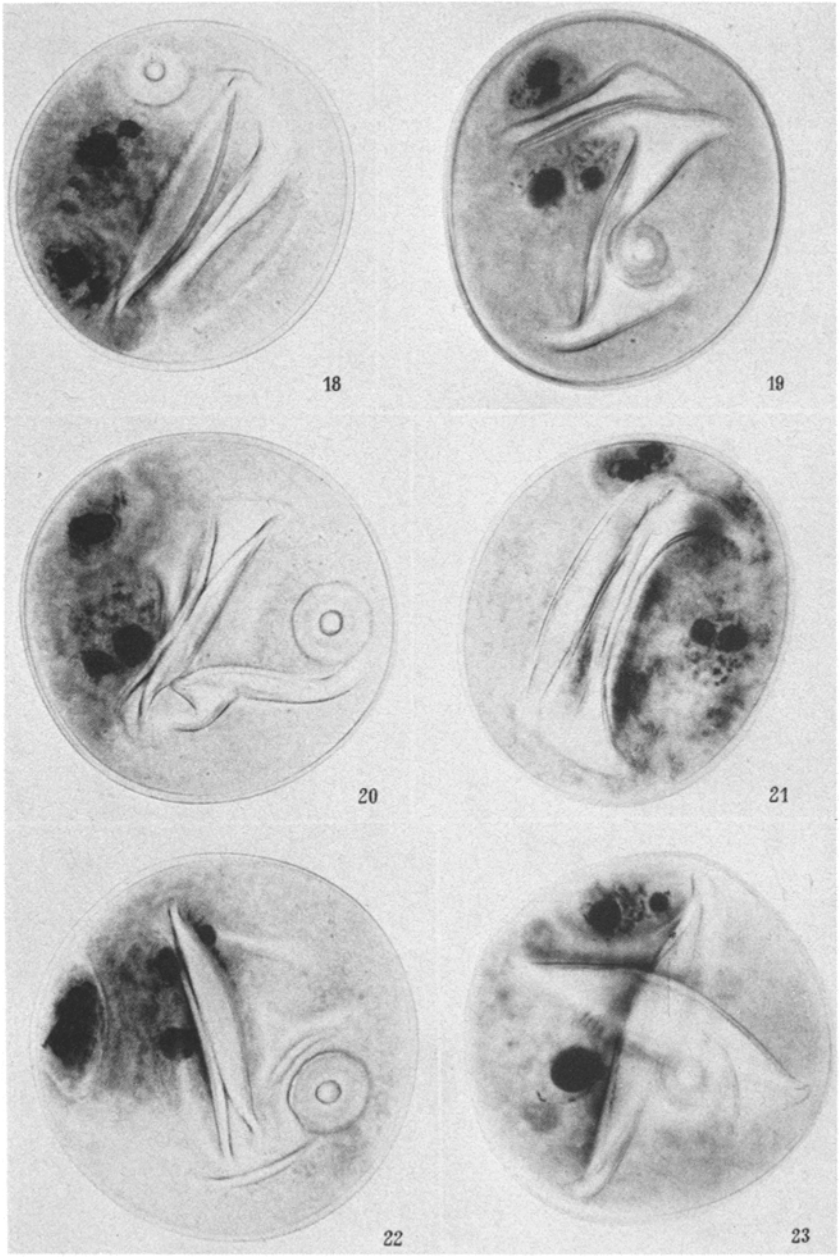
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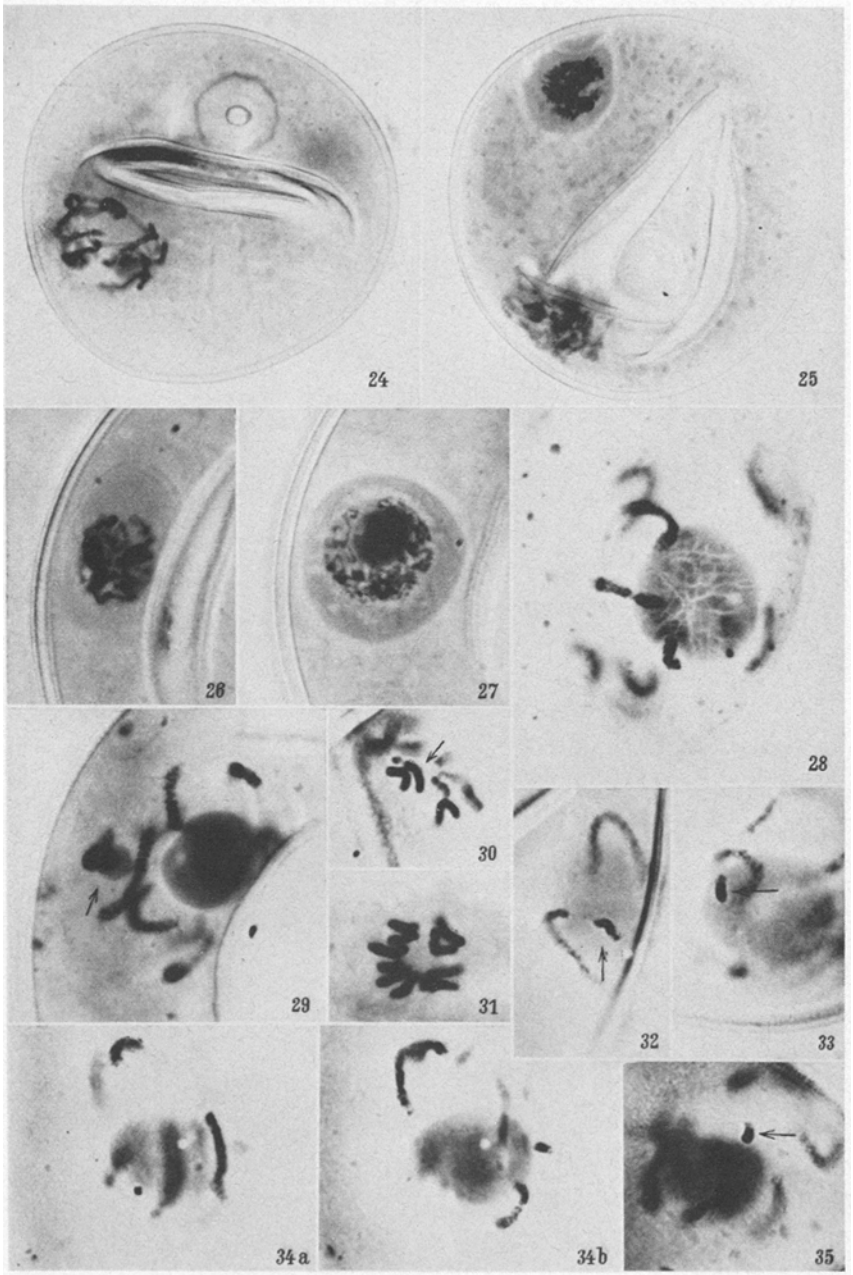
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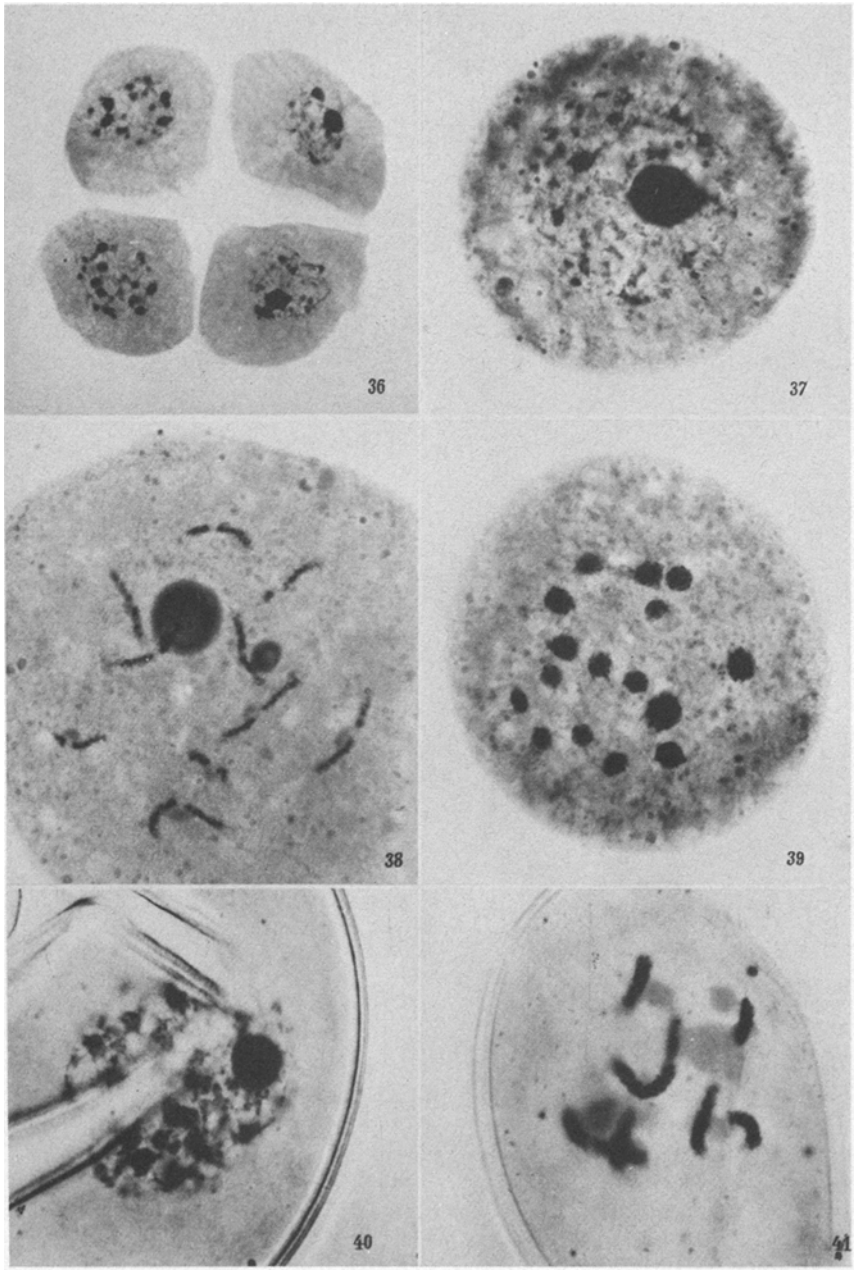
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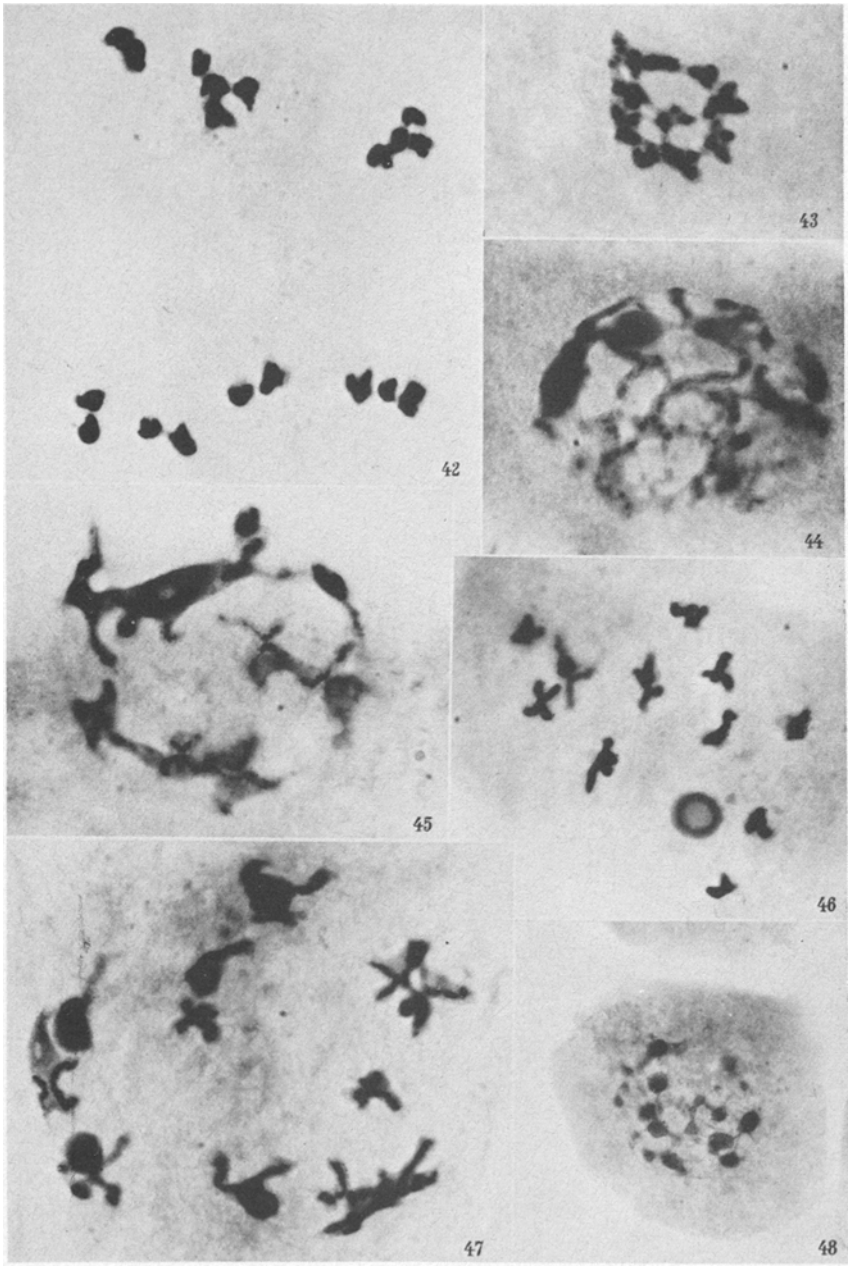
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