The Morphology of the Chromosomes of *Pisum sativum*

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Introduction

Although the species *Pisum sativum* has played an important rôle in the history of genetics and in the correlation of genetical and cytological behavior of organisms, there have been, strangely enough, but few investigations of the morphology and structure of its chromosomes. In spite of this paucity of information, repeated attempts have been made to find explanations of genetical behavior in the appearance of its chromosomes. CANNON (1) investigated sporogenous and reduction divisions in some races of *Pisum sativum* and reported the diploid number of chromosomes as fourteen, the reduced number as seven. He found no difference between the chromosomes of second generation hybrids (F₂) and those of the grandparental plants. NĚMEC (14), studying the effect of chloral hydrate on nuclear and cell divisions, figured some normal as well as treated somatic metaphases; but these showed no individual differences between chromosomes. STRASBURGER (21), dealing also with the effect of chloral hydrate on root tips, described and figured prophase chromosomes as ladder-like structures, and metaphase chromosomes showing size differences. Some of these showed spiral or ladder-like internal structure. He could not discern any individual characteristics of the chromosomes in the first meiotic metaphase. KEMP (8), studying the effects of chloral hydrate on root tips of *Vicia*, *Galtonia* and *Pisum*, noticed that "the normal pea has further a curious break at one end of its longitudinally split chromosomes." SAKAMURA (16) figured a somatic metaphase plate with a pair of chromosomes having terminally constricted portions and observed that the

1) This investigation was begun in the Dept. of Botany, Cornell University and completed at the Bussey Institution, Harvard University.

spindle fiber attachment was not at the constriction. DOMBROWSKY (4) investigated mitotic divisions in root tips of several races of peas and described some of the chromosomes as being segmented into two or three parts. One pair of the segmented chromosomes bore satellites which were attached "par des fils fins. Chaque acolyte a la forme d'un petit cylindre, fixé à sa chromosome par le milieu de sa surface, de sorte qu'il apparaît tantôt comme un petit rond tantôt comme un rectangle, plus étendu. On aperçoit ces acolytes dans tous les stades de la division. En stade de métaphase on les voit divisés de même que leurs chromosomes et les fils par lesquels ils sont fixés." Also "quelques chromosomes paraissent avoir deux têtes à l'extrémité et même en stade de peloton on peut quelquefois voir une extrémité delaminée du fil qui apparaît parfois didyme." WINGE (26) pictured meiotic metaphase plates with chromosomes as pairs of homogeneously staining short thick rods, individuals of each pair being parallel or crossed. WELLENSIEK (25) described a "clavate tumefaction" at the ends of some of the somatic metaphase chromosomes. HEITZ (7) published a figure of a root tip metaphase showing no constrictions but indicating possible size differences. LEVITSKY (11) illustrated a somatic metaphase plate in which three chromosomes were figured and described as having "satellites," two of the "satellites" being smaller than the third. HÅKANSSON (5), investigating a strain of peas which showed linkage of two genetic factors ordinarily independent, found ring formation by a pair of gemini. Further genetical evidence in favor of the ring formation as the cause of the linkage was later given by HAMMARLUND and HÅKANSSON (6). RICHARDSON (15) used a similar ring formation in meiosis to explain fifty percent sterility in hybrids of a cross between two lines of peas. A review of the literature mentioned above makes it evident that with the exception of some of the earlier workers and of HÅKANSSON and DOMBROWSKY recently, cytologists have dealt with *Pisum* only incidentally. This present work was undertaken in order to obtain more detailed information concerning the characteristics of the individual chromosomes of the complement and thereby render more probable the allocation of genes to particular chromosomes and also the possibility of finding cytological explanations for genetic behavior.

Preliminary Statement.

The investigations to be reported here fall into two distinct groups and will be so treated. First, methods of fixing and staining material

will be described and the significance of the results discussed. This will be followed by a description of the chromosomes from those preparations which were found to show the individual characteristics of these bodies most clearly. In both cases somatic chromosomes will be dealt with before meiotic.

The root tips of many different strains—tall, dwarf, early and late flowering types of garden pea (*Pisum sativum*) and also the Canada Field Pea were examined. Most of the bud material was from a variety known as Carter's Daisy or Dwarf Telephone, though Gradus and Little Marvel varieties were also used. Since no cytological difference was noted in any of these types, the particular varieties used will not be designated in the descriptions to follow. The stains used were crystal violet, methyl green, ordinary iodine green, iodine green purified by extraction with chloroform, and hæmatoxylin (with and without iron alum mordanting). The descriptions, unless otherwise designated, will refer to preparations stained with crystal violet.

All figures were drawn with the aid of an ABBE camera lucida at a magnification of 7,000 $\times$ and were reduced one half in reproduction. For critical examination, SPENCER microscope 93769 was used with the following lenses:

Objectives:—SPENCER 1.8 mm., N.A. 1.25, 1.5 mm., N.A. 1.25.

Oculars:—SPENCER 10 $\times$, ZEISS 15 $\times$, ZEISS 20 $\times$.

Methods of Fixation

Root tips.

Root tips were fixed for 24 hours in solutions made up as follows:

- 6.2 cc. water
- 0.5 cc. organic acid
- 0.8 cc. aldehyde
- 5.0 cc. of 1 percent chromic acid

Substitutions were made either of the organic acid or of the aldehyde but not of both simultaneously. For example, a series of four fixatives was made up, one of which contained acetic, another propionic, the third butyric, the fourth valeric acids, the other constituents of the solution being kept constant. A second series was made using acetic as the organic acid with different aldehydes—formaldehyde, acetaldehyde, and butyraldehyde.

After fixation, the root tips were washed in running water for 24 hours and run up in an apparatus designed to give a gradual increase

in concentration of alcohol. Then having been dehydrated in absolute alcohol they were again put into a similar apparatus in which the concentration of xylene was gradually increased. They were then placed in xylene for several hours and imbedded in paraffin.

When acetic is used as the organic acid the formula of the fixative is essentially that of NAWASCHIN and the figures obtained show the metaphase chromosomes as bent rods, one or more of which may show constrictions. The most characteristic image is found in the prophase at the "spireme stage" where the chromosomes appear as in the metaphase, homogenous and more or less round in cross section. Some seem to be plainly connected with the nucleolus which is round or ellipsoidal though sometimes amoeboid and which, in the metaphase as well as the prophase stage, destains before the chromatin (Fig. 22). When fixed the nucleolus is hard, so that if hit by the knife in sectioning it is knocked out of the nucleus without being distorted.

When propionic acid is used in place of acetic the fixation image seems to be essentially the same, while with butyric and valeric acids, the reticulum of the metaphase nucleus is more coarsely granular and the chromosomes are somewhat thicker. The fixative containing the valeric acid shows after 24 to 48 hours a violet precipitate, that is insoluble in alcohol, xylene and chloroform but is dissolved by acetone. When, with acetic as the organic acid, acetaldehyde is used in place of formaldehyde, there is no change in the appearance of the chromosomes except that they seem somewhat thicker. The nucleolus is either amoeboid or round with several small vacuoles. With butyraldehyde the image is essentially the same. The vacuoles in the nucleoli of the butyraldehyde preparation contain deeply staining granules.

Another series of fixatives contained 10 cc. 0.5 percent chromic acid and 0.20 cc aldehyde (equal parts of 0.5 percent chromic acid and 5 percent commercial formalin). The metaphase chromosome obtained with this preparation appear thinner than those previously described, show constrictions much more frequently, and are plainly double before the anaphase separation (Figs. 23, 25). The most striking difference between the image produced by this fixative and those containing organic acids is found in the spireme stage of the prophase (Figs. 20, 21). The chromosomes here are not wire-like structures, homogeneous and round in cross section, but usually appear to be constituted of a pair of thin, erratically spiral threads which may sometimes be indistinguishable in a portion of the chromosome (Fig. 21). They do not show any definite connections with the nucleolus though they may be in some

cases closely apposed to it. The ends of the chromosomes are frequently terminated by knob-like structures which approach or touch the nucleolus, but in no case is there the intimate fusion of the end of the chromosome with the nucleolus that is apparent when the material is fixed in solutions of the previous series. The nucleolus itself is strikingly different when fixed by this method. It is usually amoeboid or markedly vacuolate and retains the stain after the chromatin in the metabolic and prophase stages has lost all color. When acetaldehyde is used in place of formaldehyde the nucleolus is amoeboid or round, and the chromosomes are of the thick type. Butyraldehyde gives results like those of acetaldehyde. When the root tips are placed in 0.5 percent chromic acid for 24 hours, washed in water for half an hour, and then treated with 5 percent formalin the nucleoli are round, the chromosomes are of the thick type; and the metabolic chromatin, which usually does not retain the stain when fixed by the other methods of this series, shows as a very prominent, coarsely granular reticulum. When the material is treated first with 5 percent formalin for 24 hours and then with 0.5 percent chromic acid, the image obtained is very different—the chromosomes are thin, the nucleolus is irregular in outline, and there is no metabolic reticulum. If one percent formalin is used instead of 5 percent, the protoplasts are badly plasmolysed and appear completely disorganized—nuclei, chromosomes, and cytoplasm being indistinguishable. Variations in the period of washing after fixation with 0.5 percent chromic and 5 percent formalin, produce noticeable differences in the appearance of nucleolus and chromosomes. After twelve hours of washing the nucleoli are round and vacuolate or amoeboid, and the chromosomes are of the thin type. Material taken from the same fixative, but washed for twenty four hours, show chromosomes thinner and nucleoli more markedly amoeboid. Root tips kept in water for twelve days after fixation show nucleoli very much diminished in size and either round or amoeboid. (For washing, running water was used in all cases except the last where after one day in running water the material was placed in an ERLLENMEYER flask of water covered with a film of toluene.) Material from the same fixative washed 24 hours and then kept in 70 percent alcohol for eleven days showed no apparent decrease in the size of the nucleoli.

Smears.

The contents of young anthers were smeared on dry slides, fixed in various ways, and stained with crystal violet in the usual manner.

After two hours of fixation in a mixture of chromic and acetic acids and formalin, in the proportions used for the root tips, followed by two hours of washing, the chromosomes of the first meiotic metaphase appear, in most cases, as solid blue bodies sharply delineated from the surrounding cytoplasm by definite membranes. (The term membrane is here used in a descriptive sense to designate a clearly defined interface between two protoplasmic constituents e.g. cytoplasm and chromosomes, and not a morphologically distinct structure. The significance of the appearance will be discussed later.) Occasionally there is a suggestion of chromonemata contained within the membrane. When destained further, the chromosomes appear as vesicles containing deeply staining granules. If, after fixation, the smears are kept in absolute alcohol for a week, the chromosomes appear to be swollen but are essentially like those just described.

When the smears are immersed in 0.5 percent chromic acid for two minutes and then removed to crystal violet, only the periphery of the chromosome is stained and this colored pellicle is perforated by several large, colorless, more or less circular lacunæ (Fig. 3). The chromosomes of the late first meiotic anaphase are plainly double after all fixatives. Usually the constituent halves are widely separated, diverging from the region of spindle fiber attachment—the only place where they are in intimate contact. After chromic acid fixation, however, although the divergence from the region of spindle fiber attachment is evident, the distal ends of the chromosome halves again converge as though brought together by an enclosing membrane.

Preparations treated with forty percent formalin for three to twenty minutes and immediately immersed in the stain after rinsing in tap water, show first metaphase chromosomes as solid blue bodies which sometimes show chromonemata (Figs. 4, 5, 6). If after such a fixation the slides are washed in running water for an interval of about ten minutes before staining a greater number of chromosomes seem to show chromonemata. Long continued washing (24 hrs.) or treatment with absolute alcohol (one week) does not increase the percentage of distinguishable chromonemata. In contrast to the action of chromic acid, this method of fixation produces no definite membranes about the chromosomes. These, though distinct as bodies, are hazy in outline. If the formalin treatment is followed by immersion in 0.5 percent chromic acid, membranes are in evidence about the chromosomes and vacuoles. When treated with forty percent formalin for three minutes, followed by half percent or five percent chromic acid

for three minutes and stained immediately after rinsing, the first metaphase chromosomes are alveolated very much as after chromic acid alone. If, after such fixation, the smears are washed in water for an hour or more, there is no alveolation of the chromosomes, the cytoplasm is not vacuolated, the metabolic chromatin not clumped, and sometimes there is definition of chromonemata within the chromosomes.

Figs. 1-6. 1. First meiotic metaphase. Absolute alcohol 30 minutes. 2. First meiotic metaphase. Absolute alcohol 30 minutes. 3. First meiotic metaphase. 0.5 percent chromic acid. 4. First meiotic metaphase. 40 percent formalin. 5. Early meiotic anaphase. 40 percent formalin. 6. Early meiotic anaphase. 40 percent formalin.

The use of formalin at concentrations of five percent or less, in this procedure, produces marked vacuolation of the cytoplasm, and clumping of the chromatin in the metabolic stage. However, chromatin in the division stages is not as easily disorganized, and first metaphase chromosome frequently show chromonemata. When the material is washed several hours after such fixation and left in absolute alcohol for a day, there is no evidence of matrix or pellicle about the chromosomes, although the chromonemata are clearly outlined and deeply stained.

If the fixative is entirely omitted and the slides placed directly into the crystal violet solution for a half to several hours and destained as usual with alcohol (with or without iodine), there is little or no vacuolation of the cytoplasm or clumping of chromatin (Fig. 11). Only those portions of the protoplast which were in more immediate contact with the dye are found to be stained. For example, a cell in the late second anaphase stage commonly shows only the one or two chromosome groups at the uppermost focus, that is, the portion of the cell away from the surface of the slide and directly in contact with the dye solution. Only the external portion of chromosomes so treated is stained; and since no chromonemata are differentiated, the appearance is one of blue membranes enclosing colorless bodies whose size and shape de-

pend on the stage of division and individual characteristics of the chromosomes concerned.

When fixed for half an hour in absolute alcohol, the chromonemata in the first meiotic metaphase are usually well differentiated, while the matrix is scarcely or not at all visible, though there is a thin membrane or portion thereof marking the outline of the whole or part of the chromosome (Figs. 1, 2).

After fixation with chromic acid, chromic-formalin, and chrom-acetic-formalin, there are reticuli about metabolic nuclei which are not found after formalin or alcohol.

Whole buds were fixed in chrom-acetic-formalin, chromic-formalin, formalin, and alcohol. Only the first two fixatives gave reasonably good figures, the meiotic metaphase chromosomes appearing as seven solid blobs or rods, usually plainly double, but showing no internal structure and no individual characteristics beyond possible size differences. Since they are essentially like the published descriptions of CANNON, STRASBURGER and WINGE, no further attention will be given them here.

Discussion of Results with Fixatives

A review of the results obtained with root tips indicates a correlation in the appearances of the chromosomes and of the nucleoli after fixation. When one of the lower fatty acids is present in the fixing fluid, the nucleolus is usually more or less round, while the chromosomes in the spireme stage of the prophase are wire-like, and in the metaphase, rod-like. In neither of the two stages are chromonemata apparent. With the organic acid omitted from the fixative, the nucleolus is commonly amoeboid, and the chromosomes in the same stage of the prophase appear as pairs of chromonemata with little or no matrix evident about them. In the metaphase the chromosome matrix is still invisible, while the chromatic portion, no longer coiled or zigzag, appears considerably shorter and wider than in the early spireme stage. That the apparent absence of the nucleolar and matrix substances is due to their solution and removal is indicated by the effects of variations in the duration of the washing period. Longer immersion in water not only makes the nucleolus irregular in outline but eventually reduces its size considerably. Similarly the matrix substance disappears upon longer washing. Treatment with alcohol instead of water does not have such an effect. It seems logical to conclude, therefore, that the nucleolar and matrix substances or some of their constituents

are rendered soluble in water by the action of the fixative, or that being water soluble in the living condition are not rendered insoluble upon fixation. The swelling of meiotic chromosomes due to imbibition observed by KUWADA and SAKAMURA (9) and the solution of their matrix substance by boiling water reported by SAKAMURA (17), makes the latter alternative seem the more probable. That formaldehyde is the component of the fixative responsible for the solution of the nucleolar and matrix substances is demonstrated by treating the material with each of the different constituents alone and then following with the other. Thus chromic acid used first preserves the nucleolar and matrix substances but formalin does not. Incidentally, the theory that the first constituent of the fixative to react with the protoplast determines the type of fixation is here again demonstrated. The fixation image of chromic-formalin is essentially that of formalin and according to theories of permeability, formalin should penetrate more rapidly than chromic acid.

Since both the nucleolar and matrix elements behave similarly, it is but natural to inquire into their possible identity. NAYLOR (13) and KUWADA and SUGIMOTO (10) have shown that chromosomes may acquire a staining reaction similar to that of the nucleolus. ZIRKLE (27, 28), using a fixative (nickel-chrome-acetate) that dissolves all chromatin but preserves nucleolar material, has been able to demonstrate the entrance of some of this substance into the prophase spireme. With material fixed in chrom-valeric-formalin, stained in hæmatoxylin, and then washed in dilute alkali, it is possible to obtain violet cytoplasm, blue reticulum, and red nucleoli in the metabolic nucleus. With the development of the prophase, the reticulum and later the spireme becomes purple and more and more red until in the metaphase and anaphase the chromosomes stain the same color as the nucleolus. In the telophase the reticulum again appears blue and the nucleoli red. Although this would seem to indicate that nucleolar material enters the chromosomes, nevertheless it is possible by varying the post-staining procedure to make the chromosomes take the color not of the nucleolus, but of the cytoplasm. ZIRKLE, using iron alum hæmatoxylin after different fixatives, has described similar behavior. SCHAEDE (18) believes that there is no mass flow of nucleolar material into the spireme, though there may be fine connections between the reticulum and the nucleolus earlier in the prophase. Evidence of apparent dissimilarity of the materials composing the chromosome matrix and those of the nucleolus is found upon staining with ordinary iodine green or methyl green after chrom-acetic-formalin. Although the nucleoli may be green, colorless,

or blue—all three types may be found in a single nucleus,—the chromatin and chromosomes are blue. When the lower phenyl-methane impurities such as methyl violet are removed by extraction in chloroform, the purified dye stains all the cell constituents green. This indicates that the different protoplasmic constituents after fixation have varying, but not constantly different, affinities for the different dyes of the phenyl-methane series. Furthermore, although the matrix substance of meiotic chromosomes reacts to the fixatives very much as does that of the somatic chromosomes, no definite connections between nucleolus and chromatin—of the type found in root-tip mitoses after chrom-acetic-formalin—could be found after any of the fixatives used. The evidence from staining reactions in favor of the identity of the nucleolar and matrix materials is therefor rather weak.

There is, however, another series of facts to be considered. The fixative which preserves the nucleolar material likewise fixes the matrix substance which, in the prophase, can clearly be seen to be continuous with the nucleolus, though the chromonemata are not. Just as the problem involves the disposition of the nucleolar material in the prophase, so does it also include the origin of the nucleoli in the telophase. Since no nucleolar fragments are seen in the metaphase, and since there is no indication of extrusion of nucleolar material into the cytoplasm, it is difficult to imagine the new telophasic nucleoli as being built up from remnants of the old nucleolus. The telophase figures to be described later, suggest that the nucleoli arise at constricted portions of some of the chromosomes. Similarly, in the interphase of the meiotic division, nucleoli are seen to arise at definite loci on the chromosomes. Finally, the chromatic material, left after the matrix substance is dissolved, is in mid prophase and early telophase identical with the chromonemata. And since these, in the light of recent cytological evidence and of the present findings, are definite structures maintaining their individuality from one cell generation to another not only in the ontogeny of the individual but also through the gametic stage and thus throughout the life of the race, it seems evident that we are dealing, not with artificial appearances induced by fixation—for chromonemata have been observed in living cells—, nor with more or less transitory conditions found in a particular tissue; but that in fixation two fundamental constituents of the chromosome are segregated, one of them being in part at least of nucleolar origin. All of these observations taken together furnish rather convincing circumstantial evidence that some of the nucleolar

material may be identical with some or all of the matrix substance of mitotic chromosomes. These matrix and nucleolar substances are preserved after fixation with chromic acid and chromic-fatty acid-formalin, but not after formalin or chromic-formalin.

Similar behavior is found with smears of pollen mother cells. After chrom-acetic-formalin, the entire chromosome is preserved, externally at any rate; but chromonemata are usually not differentiated. The matrix substance here seems to be fixed but differs in its staining reaction from the matrix substance of somatic chromosomes. There is further evidence of some difference between these substances in the absence of any definite connection between the nucleolus and the meiotic prophase chromatin. There may be an interchange of materials between the two, nevertheless, since the nucleolus, upon being squeezed out of the reticulum, is usually more or less pear shaped with the end of the chromatic strand very closely approaching the apex of the nucleolus, so that it looks as though the nucleolus had drawn out with it a strand of chromatin with which it had been connected by some non-chromatic material or to which it had a very strong physical attraction. It is possible, therefore, that some transfer of materials occurs. After formalin, the matrix, or one or more of its constituents, is rendered soluble, and interfaces are not sharply defined; whereas, after chromic acid, there is little or no solution, and sharply defined membranes are found about interfaces. The stainable membrane about the chromosomes may be the product of the reaction of chromic acid with the chromosome surface, the chromic ions and molecules being held only at this surface; or else there may be an actual pellicle about the chromosome. Although the behavior of chromic acid in precipitating membranes in protoplasm, where none existed in the living stage, would lead one to suspect the chromosome pellicle of being an artifact; nevertheless, the descriptions of chromosomes in living cells particularly those of SAKAMURA and KUWADA and SAKAMURA, make the presence of a true morphological membrane very likely.

Although in root tips the mixture of chromic acid and formalin gave essentially the fixation image of formalin, with smears the same fixative gave results like that of chromic acid alone. This is easily comprehended when it is considered that with the smear preparations chromic acid and formalin are in contact with the cells at the same time, though in the root tips the formaldehyde has probably reached the cells some time before the acid. Unlike formalin, alcohol appears to dissolve the matrix substance instead of rendering it soluble to water. Since

there is no proof of actual solution, its action upon the chromosomes may be one of differential fixation of matrix and chromonema. The appearance of the chromosomes make the former alternative the more probable. The slides put directly into the stain must be considered as having been fixed in alcohol since in the destaining process they were subjected to the action of alcohol for three or four minutes.

Regardless of what the theory of the action of these fixatives may be the results obtained with each of them have been consistent, so that it has been possible to obtain interesting information concerning the morphology and structure of both somatic and meiotic chromosomes which are presently to be described.

Mitosis

No elaborate study was made of the behavior of the chromosomes in the early prophase. The procedure during this and later stages seems to be the same as that described by SHARP (19) for *Vicia*, *Allium*, and other plants with large chromosomes, with this exception: no definite chromonemata were observed within the half chromosomes of the late prophase, metaphase, and anaphase stages. A pair of chromonemata are readily visible in the mid prophase (Figs. 20, 21); but, with the shortening and thickening of the chromosome, this structure becomes indistinguishable though the two halves are clearly separated (Fig. 23). In the telophase the chromonemata are again readily discerned. Chromosomes of *Secale cereale* showed similar behavior, the two chromonemata within each chromosomal tract being particularly clear in mid prophase and telophase, but not at all evident in the intervening stages. This was true of material stained in HEIDENHAIN's iron-alum hæmatoxylin as well as of that treated with crystal violet. Although this behavior suggests a change in the matrix substance immediately surrounding the chromonemata or some change in the chromonemata themselves correlated with a possible chromonematic division at this stage, there is no direct evidence from the *Pisum* material with which to substantiate such a theory.

In the mid prophase, constrictions in the chromosomes are commonly seen. They appear more frequently in the late prophase, metaphase, and anaphase (Figs. 23, 24, 25). They are confined to particular loci on the chromosomes and, as has been frequently demonstrated in other plants, furnish dependable means for identifying the chromosomes. The most useful stage for determining the individual charac-

teristics of each of the chromosomes was found to be the very late prophase where the chromosomes had, in general appearance, reached their full development, though they had not yet become alligned on the median plate. It is difficult to obtain a good view of all of the chromosomes in either metaphase plates or side views of metaphase and anaphase figures. The difficulty with metaphase plates as revealed by side views is that only a portion of each chromosome is actually on the plate, the ends being distributed at random and commonly pointing to either pole. Anaphase figures present the usual difficulty of overlapping of chromosomes at the polar ends, though the distal ends are ordinarily well separated. In this stage the subterminal constrictions of the longer chromosomes are easily recognized. Frequently, four or five such constrictions may be observed in each anaphase group and, since the distal chromatic portions vary in size, it is evident that there are several pairs of such constricted chromosomes. In the reconstruction of the daughter nuclei chromatic globules appear at constricted portions of several of the chromosomes (Fig. 19), and seem later to fuse to form one or two nucleoli. Although it is not definitely known whether these constrictions are primary or secondary, analogy with the meiotic interphase behavior to be described indicates that probably these globules arise only at the primary constrictions.

Meiosis

No special study was made of the prophases or of diakinesis in the first meiotic division of the microspores. The metaphase chromosomes after the proper methods of fixation can be seen to contain chromonemata which are not necessarily coiled, but bent and folded in various ways. Commonly, two chromonemata are seen in each chromosome. The double nature of each chromonema is clearly evident in some regions particularly at chiasmata. (The term chiasma is here used to indicate that portion of the chromosome where there is a visible interchange of chromatids between chromonemata. Such an interchange does not necessarily result in genetical crossing-over.) Some of the chiasmata represent simple interchanges of chromatids between chromonemata that are terminalized upon the separation of the chromosomes (Figs. 7 upper and lower chromosomes, 8, sixth chromosome). Others cannot be so interpreted (Figs. 6 and 7, third and sixth chromosomes from bottom). In the best metaphase figures it is possible to see the four chromatids almost throughout the entire length of the

chromonemata. There are, in addition, chromatic strands between chromatids of different chromonemata whose significance is not understood, though in some cases they suggest a possible doubleness of the chromatid (Fig. 2). In the early anaphase, just before they are sepa-

Figs. 7-11. 7. Early meiotic anaphase. Absolute alcohol. 8. Early meiotic anaphase. 40 percent formalin followed by 5 percent chromic acid. 9. Mid meiotic anaphase. Absolute alcohol 30 minutes. 10. Mid meiotic anaphase. Absolute alcohol 30 minutes. 11. Mid meiotic anaphase. Stained in 1 percent crystal violet without previous fixation. Overlapping made chromosomes of upper group indistinguishable individually.

rated, some of the chromosomes show characteristic configurations which can be identified with some of those in the published figures of HÅKANSSON and RICHARDSON (Fig. 7, second chromosome, Fig. 8, third chromosome). Immediately after the chromosomes have separated, each half chromosome appears strikingly like its somatic homologue (Figs. 9, 10). There is an elongation of the chromosomes in the early anaphase followed by a shortening, and in some cases a coiling, when

or shortly before they reach the poles (Figs. 12, 13). With the onset of the interphase the chromosomes again elongate and a nuclear membrane is formed. After fixation with alcohol, interphase chromosomes gradually lose their chromaticity throughout their entire length with the exception of the region of spindle fiber attachment. These areas remain prominently chromatic when the chromosomes themselves are barely distinguishable (Fig. 17). After chromic-formalin, the chromosomes do not become achromatic but appear as threads which become increasingly finer as the interphase progresses. The regions of spindle fiber attachment in early interphase appear as chromatic globules very

Figs. 12-20. 12. Late meiotic anaphase. View from pole to plate. Absolute alcohol 30 minutes. 13. Late meiotic anaphase. View from plate to pole. Absolute alcohol 30 minutes. 14. Second meiotic metaphase. Absolute alcohol 30 minutes. 15. Second meiotic anaphase. View from plate to pole. Absolute alcohol 30 minutes. 16. Second meiotic anaphase. View from pole to plate. From the same cell as 15. 17. Meiotic interphase. Absolute alcohol 30 minutes. 18. Nucleolar-like globule, one of seven from an interphase nucleus. Chromic-formalin. 19. Somatic interphase. Nucleolar globules at chromosome constrictions. Chromic-formalin. 20. Somatic prophase. *a.* upper focus, *b.* lower focus of same nucleolus. Chromic-formalin.

much like small nucleoli (Fig. 18). Later, there are four or five and finally two nucleoli that are proportionately larger, suggesting that they may have been formed by fusion of the smaller ones. At the stage when there are usually two large nucleoli the chromosomes appear very much as they do in the spireme stage of a somatic prophase, the half chromosomes being in close juxtaposition though in the previous anaphase and early interphase they were widely separated. In the second metaphase the subterminal constrictions are again evident and it is possible to identify some of the chromosomes though this is much more easily done in the previous or succeeding anaphases.

Chromosomes

Because of the confusion which exists with regard to the interpretation of chromosome structures it seems necessary to make some definitions before proceeding with the descriptions. The types of morphological chromosome characters have been classified by TAYLOR (23) as: 1. constrictions directly concerned with the position of attachment of the spindle fiber; 2. constrictions unrelated to the fiber attachment; and 3. comparatively small bodies attached to a chromosome by a cord of considerable length, the length of the cord being equal to or greater than the diameter of the body itself. DELAUNAY (3) has made a similar classification and has used the terms kinetic or primary constriction to refer to the first, akinetic or secondary constriction for the second, and satellite for the third. The distinction between the first and second categories is clear, but this is not the case for the second and third as TAYLOR (24) has shown; for conditions intermediate between the two exist. The secondary constriction may be relatively long and the terminal chromatic segment very short, simulating the satellite and its "cord" or "Faden". Because of this difficulty it seems advisable to confine the term satellite to bodies picked up from the nucleolus by a fine thread attached to the prophase chromosome as described by NAWASCHIN (12). It does not seem wise to use the terms monobrachial and dibrachial as suggested by SOROKIN to designate chromosomes with one or two arms, since, as DELAUNAY has pointed out, a chromosome in the metaphase may not be at all bent at the kinetic constriction but may be markedly bent at some other region. Similarly it does not seem advisable to use Roman letters to describe chromosomes as J-shaped or V-shaped, since the configuration of particular chromosomes are not confined to limitations set by that or any other alphabet.

There are in *Pisum sativum* no satellites as defined by S. NAWASCHIN, since there are no bodies picked up from the prophase nucleolus by a thread on one or more of the chromosomes. After chromic-formalin fixation, some of the somatic prophase chromosomes are terminated by one or more round globules (Fig. 20). Although they are frequently on or near the nucleolus they do not show the appearance or behavior of the satellites of *Galtonia* and *Muscari* described by NAWASCHIN. Their nature and significance is undetermined. If the "faden" of DELAUNAY is used as a criterion, the subterminally constricted chromosomal portions may be considered satellites as indeed they have been

Figs. 21-26. 21. Portion of somatic prophase chromosome. Chromic-formalin. 22. Somatic prophase. Chromic-propionic-formalin. 23. Very late somatic prophase. Chromic-formalin. 24. Somatic anaphase. Chromic-formalin. 25. Chromosome homologous to 26-1 and 1'. Chromic-formalin. 26. Chromosomes from figure 23.

termed by LEVITSKY (11). The constrictions when seen in face view appear as achromatic areas extending across the chromosome, usually bounded at each end by a fine chromatic membrane (Figs. 10 No. 1, 25). Seen from the side the same constriction appears as a region where the chromatic material of the chromosome tapers down and is continued only by a fine thread or not at all (Figs. 24, 26.) Consequently, although the use of the term satellite may be correct by DELAUNAY's criteria, such use leads to confusion and seems unjustifiable since the original definition of NAWASCHIN is not satisfied.

The relative lengths of the chromosomes are readily recognized in all stages with the exception of the first meiotic metaphase. They are approximately 4.4, 4.0, 3.3, 2.8, 2.5, 1.7, 1.0. In the first meiotic anaphase irregularities in this relationship may be introduced by the fact that some of the chromosomes are still stretched while others have begun to contract. The other characteristics of chromosome morphology, the number and position of constrictions, are more dependable. To the following descriptions of each of the chromosomes, a number will be appended, which will be used when it is found necessary to refer to that particular chromosome again. They are given in the order of the relative lengths of the chromosomes.

1. The primary constriction of the longest chromosome is not quite median, the arm containing the secondary constriction being the shorter. The terminal segment i.e., the segment distal to the secondary constriction, is shorter than either of the other segments of the same chromosome but is larger than the terminal segments of any of the other chromosomes (Figs. 26, 25, 15, 11, 10, 9).

2. The next longest chromosome is also divided into unequal chromatic portions by the primary constriction. Both of these segments contain subterminal secondary constrictions, the terminal chromatic portion on the longer segment being somewhat larger than the other.

3. The primary constriction divides this chromosome into a short and a long segment, the latter bearing a secondary subterminal constriction.

4. This chromosome is likewise divided into short and long chromatic segments which do not differ in length as markedly as the previous. There are no secondary constrictions.

5. The primary constriction is so located that the segment bearing the secondary constriction is shorter than the other.

6. Although easily identified the morphology of this chromosome is difficult to interpret. There is a subterminal constriction which

appears to be primary. There are in addition achromatic areas which in some cases appear to be true secondary constrictions but in others simply an illusion produced by the partial separation and twisting of the chromosome halves.

7. The primary constriction of the smallest chromosome of the complement is so located that the length of the segments are approximately in the proportion of 2:3. There are no secondary constrictions.

In a single first meiotic metaphase group five of the seven chromosomes were found to have subterminal constrictions, one of these chromosomes having two such constrictions, with the terminal portions differing in size. The terminal portion of one of the constricted chromosomes is markedly larger than any of the others (Fig. 1). This presents a striking similarity to the five subterminal constrictions in somatic chromosomes that can hardly be considered as simply a matter of chance. When the chromosomes have separated in the first anaphase, the homology of each half chromosome i.e. each metaphase chromatid, with a corresponding member of the somatic complement becomes more apparent. It is possible in such anaphases to identify all the chromosomes with their somatic homologues (Figs. 9, 10). After they have reached the poles it becomes more difficult to distinguish their individual characteristics, and it is not until the second anaphase that the morphology peculiar to each one again becomes clear. In the second metaphase, secondary constrictions are evident; but, usually, there is too much crowding and overlapping to make a sure determination of the individual chromosome (Fig 14). Knowing their characteristics, it is possible, therefore, to identify, some or all of the chromosomes in the different meiotic stages with the members of the somatic complement. Each meiotic chromonema is recognizable as the equivalent of a particular somatic prophase and metaphase chromosome, the chromatid being analogous to the half chromosome in these stages or to the daughter chromosome of the anaphase. Whether the chromatid is itself of a double nature as is the anaphase chromosome is difficult to determine. The chromatic strands between metaphase chromatids suggest such a possibility.

In the early first meiotic anaphase, it is clear that the spindle fiber attachments of the various chromosomes are at the same loci as the kinetic constrictions for the corresponding somatic chromosomes. Although primary constrictions were not observed in the meiotic metaphase chromonemata, this is hardly an indication of their absence, for the bending and coiling of the chromonemata makes the

chances of seeing such constrictions very small. The interchange of chromatids and the bending and coiling of the chromonemata often combine to make the meiotic metaphase chromosome an intricate complex. In the anaphase separation there is indication of considerable tension on the chromosome halves, exercised through the loci of spindle fiber attachments. In view of these facts, DARLINGTON's contention that the primary constriction may be simply a weak spot where the chromosome bends when it responds to a generalized attractive force seems untenable, unless one assumes that there is an essential difference between the mechanisms of meiotic and mitotic separations. This is of course possible but is an assumption that is not at all warranted by the facts of the case.

The chromosome most easily identified and which has been the first to be recognized as having a subterminal constriction is 1. The other "satellited" chromosome noticed by LEVITSKY is probably 3. None of the characteristics of the other chromosomes have been noticed hitherto. Of particular interest is 2, since it has two secondary constrictions. Although this indicates that there may be no definite limitation to the number of secondary constrictions, it offers no information as to the significance of these structures. Knowing the morphology of each of the chromosomes, it should be a relatively simple matter definitely to assign a pair of genes to a particular pair of chromosomes that show the ring formation described by HÅKANSSON; and, using some of the methods here described, it should be possible to find a cytological explanation for such behavior. A knowledge of the characteristics peculiar to each member of the complement should furnish a more useful cytological background for determining possible correlations between genetical and cytological behavior.

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Summary

1. Different methods of fixing root tips and pollen mother cell smears of peas are described and discussed.
2. Formalin appears to render some of the nucleolar material and the matrix substance of somatic and meiotic chromosomes soluble in

water, or to fail to render them insoluble as do chromic-fatty acid-formalin fixatives.

3. Alcohol appears to dissolve the meiotic chromosome matrix or to render it achromatic.

4. An intimate relationship between, and possible identity of nucleolar material and the matrix substance of somatic chromosomes is established.

5. The mitotic and meiotic chromosomes are described. The characters of each of the seven pairs permit identification of the individual members of the complement. They are as follows:

	Relative length	Primary Constriction	Secondary Constrictions
(1)	4.4	Not median	(1) Subterminal, on short segment.
(2)	4.0	„ „	(2) Subterminal, on long and short segments.
(3)	3.3	„ „	(3) Subterminal, on long segment.
(4)	2.8	„ „	(4) None.
(5)	2.5	„ „	(5) Subterminal, on short segment.
(6)	1.7	Subterminal	(6) Probably none.
(7)	1.1	Not median	(7) None.

6. The chromonemata of the first meiotic metaphase and the chromosomes of later stages are found to show the same individual characteristics as the somatic chromosomes.

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