

CHROMOSOME ABERRATIONS IN GALTONIA CANDICANS

by

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CHROMOSOME ABERRATIONS IN GALTONIA CANDICANS

Introduction

The study of aberrations in chromosome structure and behaviour has become an important foundation for the understanding of the progress of evolution. Structural hybridity resulting from the fragmentation of a single chromosome to form two, or the terminal fusion of two chromosomes with the accompanying loss of the centromere of one, may cause considerable variation in the progeny.

Each species is characterized by its own genotype, and the gene complement changes in the course of evolution may be caused by the inherent properties of the genes themselves, or by their reactions to the environment. In conjunction with such genic or genotypic changes, chromosomes are subject to another kind of changes described as structural or numerical according to whether it involves the structure of the chromosomes or their number.

In recent years a large volume of work has been done on structural and numerical changes in the chromosome complements of both plants and animals. This includes both studies of changes occurring in nature and those

induced by artificial means.

Induced aberrations have been brought about by treatment with X-rays, ultra-violet radiation, high or low temperatures, certain drugs such as colchicine, and by artificial hybridization.

A distinct separation cannot be made between artificially induced aberrations and naturally occurring aberrations because several factors used in experimental work also occur in nature, namely - variations in temperature and ultra-violet light. Age has also been found to produce alterations in chromosome structures. Moreover, induced mutations have the same phenotypic expressions and chromosomal aberrations as are found in "naturally occurring" mutations. That is, extreme conditions change the time rate of mutation rather than acting as an initial cause of mutation,

The types of changes in the chromosome complements found by recent workers include polyploidy, polysomaty, doubling or "pairing" of chromosomes, the formation of loops, fragmentation, and catenations.

The purpose of this paper is to describe the chromosomal aberrations found in untreated plants of *Galtonia candicans*, and to correlate these, if possible, with the findings of other investigators.

Materials and Methods

Galtonia candicans, Decne., is a native of South Africa, belonging to the family Liliaceae. The material used for this thesis was collected from plants growing in the Botanical Gardens at the University of British Columbia.

Ovules of various stages were collected from several plants. No attempt was made to separate and identify the ovules as coming from different plants as there were no morphological differences noticed in the plants. Collections were made on July 14th 1941, and at about the same date in 1942. All collections were made between noon and three o'clock.

The ovules were fixed in B.C. fixing solution (95 per cent alcohol - 250 ccs., glacial acetic acid - 5 ccs., formalin - 12 ccs., water - 50 ccs.) This is a successful fixative because the tissues may be left in it almost indefinitely without causing excess dehydration or malformation. The usual dehydrating and embedding procedures were followed using methyl alcohol and xylol as the clearing agent. The material was embedded in paraffin and sections were cut at about 12 microns.

Egg albumen was used as an adhesive when the sections were to be treated with Feulgen's technique because it was found that the sections had less tendency to float off the slides during the hydrolysing process than when the gum arabic - potassium dichromate adhesive was used. However this latter adhesive was found to be more satisfactory than egg albumen for the other staining techniques used.

Several stain combinations were tried. Firstly, Flemming's triple stain (safranin in 50 per cent alcohol, gentian violet aqueous, orange G aqueous) plus light green in clove oil - this combination stains the chromosomes purple, the nucleolus bright red, and the spindle fibers green. It is not very satisfactory for detailed work. The second stain was iron-alum haematoxylin with or without a counter stain of light green. The chromosomes are stained dark blue or black. The difficulty with this stain is that it stains the nucleolus the same as the chromosomes, and also unless it is destained extensively no details of the internal structure of the chromosome can be seen. The third technique used was the Feulgen method ((fuchsin sulphurous acid) followed by a light green nucleolar counter stain. It was the

most satisfactory combination used, as it differentiated the nucleolar material from the chromatin and was best for showing the internal structure of the chromosomes.

The root tips were obtained by germinating *Galtonia* seeds which were collected on October 1st 1941. Some of these seeds were incubated in petri dishes between moist pieces of filter paper at room temperature the week following the collection. These were fixed in B.C. fixing solution, embedded in paraffin, sectioned at 10 microns, and stained by the Feulgen method. Other seeds collected on the same day were incubated on March 12th 1943. These seeds were placed in constant temperature chambers at 22° C. and 29° C. Root tips sufficiently long for the experiment were produced in from six to ten days and germination was almost 100 per cent. These root tips were treated first with La Cour's fixing solution (absolute alcohol and glacial acetic acid) for about five minutes and were then transferred to B.C. fixing solution. The Feulgen's squash technique for root tips as described by Hillary (1940) was used.

Slides of the anthers prepared by Miss Lois M. Still for the Department of Botany were used for the meiotic

divisions. These slides were stained with iron-alum
haematoxylin.

Observations

Meiotic Chromosomes in Anthers

The stages of meiosis were examined carefully, but since the findings were similar to those of Smith (1932), they will not be repeated here. However, since the individuality of the chromosome pairs is apparent at diakinesis these will be recorded.

Unfortunately, since these drawings were made from sections and not smears, not all pairs were from one cell and it is possible that they are not all in the same stage of contraction. The separate pairs have been numbered for convenience (Fig. 1) ranging from longest to shortest.

The first pair are connected at both ends and often show the twist illustrated; this does not seem to be a chiasma however because the chromosomes are sometimes found forming a circle like the second pair (Fig. 2). This is the only pair in which the spindle attachment could be clearly seen. The second pair is about the same length as the first, the chromosomes are always arranged to form an O. The third pair is slightly shorter and has one terminal chiasma, the chromosomes form the arms of a V. The fourth pair is about the

same length as the third, the chromosomes are closely associated for about one third of their length at one end and then diverge, the other ends usually approximate again to form a "wish-bone". The fifth pair is the satellite pair, these chromosomes are rod-like, about half the length of the long chromosomes, and are usually attached to the nucleolus. These chromosomes themselves are seldom joined, they may lie farther apart or closer together on the nucleolus than those shown. The homologous chromosomes of the smaller pairs are not in contact at diakinesis but are approximated at regular distances. The sixth pair is similar in size and looks like two short rods. The chromosomes of the seventh pair are ovoid and those of the eighth pair are spherical.

No details of internal structure could be seen because of the very dense haematoxylin stain, even the chromatids of the bivalents were indistinguishable in most of the chromosomes.

Digby (1910) and Mottier (1907) both reported catenations or chains forming at diakinesis, but nothing of this sort was found in the material used for this paper. The only arrangement which might have been mistaken for catenation is shown in Figure 2. This is

the first pair of chromosomes forming a wide ring. The other chromosome pair, on the right side, is not connected to the first pair but lies just above it. The fact that the chromosomes are situated around the edge of the nucleus may make them appear to be joined end to end. Smith (1932) also failed to find any catenations.

Before studying the unusual mitotic chromosome behaviour in this form, the normal occurrences should be understood. Most of the root tip cells show typical mitosis stages and these stages will be discussed first.

Normal Chromosome Behaviour

Metaphase:

A polar view of the metaphase plate shows 16 distinct chromosomes. (Fig. 3) Four pair of these are long, two pair medium, and two pair short. These can be identified again at diakinesis. Since some of the chromosomes are long, they cannot lie completely on the metaphase plate, and the consequent curling makes it very difficult to measure them. In Figure 3, several of the chromosomes that appear to be of medium length are in reality long chromosomes curled up. Comparing a lateral view of the metaphase plate (Fig. 5)

with the polar view gives a better picture of the comparative length of the chromosomes.

All the chromosomes have sub-terminal spindle attachments, and the long chromosomes lie with their attachment points towards the middle of the spindle. The small chromosomes are located centrally on the plate. There is no association of homologous or even similar pairs of chromosomes at any stage in the root tip mitosis, the chromosomes are distributed quite at random. One pair of medium sized chromosomes has small terminally attached satellites. These satellites may be seen best in the prophases (Fig. 8) because when the chromosomes are contracted at metaphase the satellite is in contact with the body of the chromosome and becomes indistinguishable.

At metaphase the chromosomes are split into two chromatids but the spindle attachment is still single. The chromatids may be somewhat relationally twisted at this phase, but the coiling is not extensive. Those chromosomes in Figure 1 which do not show the division are probably lying so that the split is in the plane of the equator and would therefore not show in a polar view.

Anaphase and telophase:

Not all of the chromosomes split at the same time, in Figure 5, the chromosome on the left has split completely but has not separated whereas the upper one on the right side has separated a considerable distance. The short arm of the upper one of the two daughter chromosomes is slightly enlarged and appears to be double. This might indicate that the next split has taken place or is taking place. No internal coils could be seen in the daughter chromosomes, and no further evidence of the next split could be found. In later anaphase (Fig. 6) the chromosomes show a structure which might be interpreted either as two relationally coiled spirals or as two independent spirals lying side by side. Since the telophase (Fig. 7) shows definitely interlocking spirals, it is likely that the anaphase structure is similar.

Evidence has been found that the organization of the nucleolus commences before the end of the anaphase. One anaphase, a little later than the stage of Figure 6, showed, when stained with Flemming's triple stain, a small vacuole-like body, giving a decided nucleolar reaction, associated with one of the chromosomes. It

was not possible to see the chromosome to which this was attached because of the close association of the chromosomes at this stage.

Prophase:

The early prophases in the root tips show two definite strands or chromonemata which usually have a relic coil. This coil straightens out in the middle prophase and the strands lie parallel for most of their length. (Fig. 8) The chromonemata then shorten and thicken and a matrix becomes evident around each one. This dense matrix obscures the internal structure of the chromatids and may make the split less apparent.

Nucleoli:

In the resting stages and in the early prophases there is either usually one large nucleolus, or occasionally two small nucleoli. The large nucleolus is usually about twice the size of the small ones and probably results from a fusion of the latter.

Aberrations

The aberrations found in the somatic cells were of many types including fragmentation, pairing, fusions

and somatic bridges, chromatid fragmentations and loop formations, and polysomaty.

Aberrations in root tips - fragmentation:

Aberrations of any kind were very uncommon in the root tips. Lagging chromosomes were found quite frequently (Fig. 7) but these are probably simply the ends of some of the slower moving long chromosomes. The only example of a definite abnormality occurring in the root tips is that shown in Figure 9. This shows a stage between the anaphase and early telophase with a chromosome still on the spindle. This chromosome or chromosome fragment has two chromatids which have not separated. This is probably one of the medium lengthed chromosomes, but it has become stretched by the lengthening of the spindle. The chromatids are narrower than those at metaphase, but are not sufficiently narrow to be regarded as chromonemata of the anaphase chromosome. This chromosome appears to be either a telocentric or an acentric fragment resulting from the misdivision of the centromere. (Darlington, 1940)

Aberrations in ovule cells:

Pairing. Taking the typical root mitosis as the normal mitotic division, the first difference noticed in

the ovule cells is the side by side pairing of the chromosomes.

In the prophase the split between the two chromonemata is evident only in part, there is no complete separation of these structures similar to that found in the root tips. Figure 10 shows an early prophase with pairs of chromosomes coiled loosely around each other. I have called these threads separate chromosomes because they are much farther apart than any chromonemata found in the ovule cells, and because there were so few threads present (probably eight pairs). If each chromosome consisted of two chromonemata as widely separated as these are, one would expect the nucleus to show many more threads than it does, because there would be thirty-six of them present.

Figure 4 is a polar view of a metaphase plate, it shows definite pairing of similar, if not homologous chromosomes. The sixteen chromosomes can be seen quite plainly, two of the small ones are located under a curled pair. There are two long chromosomes that are not paired, and this same condition has been found in other cells in this ovule tissue. The chromosomes themselves do not show a split as definite as do those of the root tips;

however some of them are split in the short arm. Another polar view of a similar stage (Fig. 11) shows chromosomes that look characteristically meiotic rather than mitotic. They are more contracted than ordinary mitotic chromosomes (compare Fig. 3.) The presence of nucleoli at this stage also makes it more like the meiotic prophase, as the nucleolus is often still present at diakinesis. The pairing here, as in Figure 4, is not complete; but in this instance, one of the medium sized pairs, the satellite chromosomes, is separate. This would seem to indicate that the pairing is not strictly synaptic, but is rather a more loose association of chromosomes.

Terminal fusions. Terminal fusions of chromosomes were quite common in this material and seemed to be of two types.

The first type shows an attraction between the ends of homologous chromosomes. The attraction may be between the ends of the long arms or the short arms but not both as ring formations were not found. Figure 12 shows terminal fusions of chromosomes in the prophase. Figure 13 illustrates the fusion of the ends of the arms of two pairs of long chromosomes. In Figure 14 there appears to

be a joining of two chromosomes in the region of the spindle attachment. Two rod chromosomes appear to be attached to a single spindle attachment, forming a long chromosome with a median spindle attachment. This could not be verified because no fragments were seen and no M chromosomes were found in any other anaphase. This type of chromosome could have arisen by the loss of the short arm on one chromosome and the short arm and centromere on the other and a fusion of the broken ends, in other words, by a misdivision of two chromosomes followed by the formation of isochromosomes. (Darlington, 1940.)

The second type of terminal fusions involves the sticking together of the chromosome sheaths or pellicles. The chromosomes have become more viscous and tend to lose their characteristic shape when subjected to the forces of the spindle. Figure 15, a late prophase, shows several chromosomes attached by processes extending from chromosome to chromosome. The attachment in this case is not necessarily terminal and does not appear to be an association of homologous chromatic units. The chromosomes here are contracted to a greater extent than in ordinary mitotic chromosomes. Some of these chromosomes may be

paired parasynaptically as well as the obvious terminal association; the fact that one chromosome at the lower end of the group seems to be situated between two chromatids or chromosomes would indicate that the paired relation results from an approximation of chromosomes and not as a result of the separation of chromonemata. Figure 16 is a similar stage also showing several chromosomes attached to each other more or less at random. The left side of this group looks almost as though it had been semi-fluid and the chromosomes had flowed together.

A metaphase illustrating this viscosity and the tendency of the chromosomes to adhere to each other is shown in Figure 17. The chromosomes have become very much lumped together and non-homologous ones have become attached the one to the other at the ends. The anaphases resulting from the separation of these chromosomes have irregular chromosome bridges. Figure 18 pictures an anaphase before the spindle has expanded fully, the chromosomes are irregular but not greatly stretched. The joining of the ends of non-homologous chromosomes may be seen very distinctly here. A later anaphase, after the spindle has elongated (Fig. 19) shows long chromatin

bridges which extend from one chromosome group to the other. Very little internal detail can be seen in these chromosomes except that they are double. Alternately, it is possible that there is a failure of separation of the chromatids in these nuclei, resulting in an irregular segregation of genes. This would explain the doubleness in the anaphase chromosomes and the appearance of so few chromosomes in these divisions. The long thin chromatin bridges are probably caused by the uncoiling of the chromonemata in the anaphase chromosomes, when the spindle is stretched.

Chromatid fragmentations and loop formations. Only one definite example of a loop formation caused by a break in a single chromatid was found. This (Fig. 20) is a chromosome from the nucleus shown in Figure 12. The chromosome is located underneath the nucleolus and seems to circle the base of this structure. It was impossible to be positive whether or not this chromosome was actually attached to the nucleolus. A chromosome with a similar terminal loop was found in another nucleus, and this second one was found to be quite separate from the nucleolus. If this chromosome is the second satellite chromosome it evidently has another chromosome attached terminally; this can be seen by comparing the length of this chromosome with

the satellite chromosome attached to the left side of the nucleolus. If this configuration is the result of the fusion of two chromosomes it may have resulted in one of two ways: firstly, there may have been an internal loss in one chromatid by fragmentation, then the extra piece in the other chromatid would form a loop, or secondly, it may have arisen from the addition of a fragment forming a loop. If on the other hand, this is all one chromosome, then one of the chromatids has fragmented and one end has folded back and become attached forming a loop. In this event, there would be no loss or gain of chromatic material in the chromosome. This second explanation seems to be more likely, since both ends of the chromosome are thick and show a double structure, whereas the middle portion is narrower and no split could be seen. This cannot be decided for certainty because the chromosome is small and is located under other structures and is in consequence not entirely discrete² in appearance. -

Polysomaty. Polysomaty is the occurrence of nuclei containing multiples of the diploid number of chromosomes located in individual cells or groups of cells in a diploid organism. This condition was found in two cells located close to the outer layer of the ovule. These cells were

larger than most of the cells showing divisions in the ovule. Many of the surrounding cells were about the same size but they were all in the resting stage so there was no way to determine whether or not they were polysomatic. Figures 21a and 21b are two sections of the same nucleus showing this condition.

Most of the chromosomes are arranged in pairs, however the smallest ones have either split and separated or else they have approximated. From the number of them present it seems likely that they have separated. The longer chromosomes have split completely but most of them remain close together. Some of them are in contact only at one end, others remain coiled about each other. All chromosomes show a second partition, but the chromatids have remained more closely associated and coiled than the chromosomes formed by the first partition. The partition is not complete in some chromosomes, but may be in others, therefore the nucleus cannot be said to have octoploid chromonemata. The fact that most of the pairs of chromosomes are so closely associated would suggest that the tetraploid condition arose from a complete split in the diploid chromosomes followed by a split of each daughter chromosome to form two chromatids. The short chromosomes may have separated

quite easily because they can move in a smaller space and are less likely to remain coiled together than long chromosomes. Alternately, the nucleus originated from the fusion of two diploid nuclei, the occurrence of pairs would mean that there is no synaptic attraction between the chromosomes; we would then expect the smaller chromosomes that are able to move about most readily to form the closest associations. The fact that some of the chromosomes are separated completely or into wide V's would indicate that there is no synaptic attraction and that the twisting is merely a relic of the anaphase coil. However, since the synaptic attraction is present chiefly in the early prophases of meiosis and seems to disappear to a considerable extent by late prophase or diakinesis (see Fig. 1) and since it is less evident in the small chromosomes, the above statement must be modified. The question of attractions and repulsions is not yet clearly understood and consequently no definite conclusions can be reached.

Review of Literature and Discussion

Historical

The work done to date on the cytology of Galtonia candicans has been mainly that of tracing the chromosomes through the various stages of mitosis and meiosis. Digby (1910) has given an extended description of the minute structure of the chromosomes especially through telophases and prophases. However her work has been modified considerably since then by later workers applying more recent conceptions of chromosome structure and possibly using improved fixation and staining methods on their material. The only unusual occurrence mentioned by Miss Digby in this paper is the occasional formation of chains or rings of chromosomes at diakinesis. An earlier paper by this worker (Digby, 1909) reports the formation of chromatin bodies produced at synapsis in the pollen mother cells. These she reports as arising from the nucleolus or the nucleolar framework. Nothing of this nature has been found in the present study.

Newton (1924) in studying the somatic chromosomes in Galtonia found a loosepairing of somatic chromosomes at early metaphase and a true pairing at early anaphase.

Examining the somatic cells of the root tip and

anther, Smith (1932) made a comprehensive study of the chromonemata in the chromosome at meiosis and mitosis. He found that the chromonemata were closely associated in the anaphase of the root tips, but were more widely separated in the same stage in the somatic cells of the anther. My results indicate that there are two definite strands present even in the root tip anaphases. Smith found chromosome lagging but it was not characteristic of any particular chromosomes, he found that either long or short ones might lag. He also reports that there is no chain formation at diakinesis. The same author (Smith, 1932) working on the relation of the nucleolus and satellites found one or two nucleoli present in somatic cells with satellite chromosomes attached. In meiotic stages he finds just one nucleolus on which there may be buds girdled by the fibers of the satellite attachments.

Chromosome Structure and Spirals

Some of the recent workers have devoted themselves almost entirely to discovering the internal structure and spiralization of chromosomes. It is now generally accepted that the somatic anaphase chromosomes consist of at least two spirally wound chromonemata. Abraham (1939) reports that the somatic anaphase chromosome shows two spiral

chromonemata embedded in a common matrix, these coils are entirely free from each other and usually run parallel. The figures he shows in support of this idea are not very clear, and it seems he may have misinterpreted what his slides show. He concludes that the mechanism of spiralization is associated with a compensating internal twist in the chromonemata. Darlington (1932) refers to this twist as a molecular spiral. Abraham says that an interlaced spiral is not a result of the cleavage of a spiral chromatid but that the twists are formed later.

Coleman (1940) on the other hand agrees with Sax, Upcott, and Darlington in regarding the somatic anaphase and prophase chromosomes as single coiled structures. He states that the so-called interlocking double coiled structure is a fixation artifact. He further states that this conclusion does not touch the question as ^{to} whether the anaphase chromosomes are bipartite or not. From this I gathered he means that there is only one spiral but it might be double.

The great majority of cytologists consider that the anaphase chromosomes possess a visibly bipartite structure and that they consist of two chromonemata coiled about each other so that the gyres interlock.

Wilson and Huskins (1939) working with Trillium propose the theory that the meiotic spiral is caused by the elongation of the chromonemata within an enveloping sheath or pellicle, the nature of which remains to be determined. Sparrow, Huskins, and Wilson (1941) add that the half chromatids at metaphase are wound in the form of a plectonemic spiral. This is a spiral arranged so that the chromatids can separate by lateral movement and not by unravelling from the ends. Nebel (1941) presents interesting optical evidence of the types of coils found in Trillium chromosomes, he photographs light transmitted through glass spirals and compares the results with the images formed by the chromonemata coils. Huskins (1941) describes the large gyred major coils in meiosis, and similar coils in mitotic chromosomes. The evidence presented in these papers was taken from material prepared especially to show these structures. The spirals that show in my material are not nearly so clear as the illustrations shown by these men, but they do indicate a similar structure, namely, a pair of spiral chromonemata in the anaphase chromosomes.

The time of the somatic split has been found to vary in different forms. Nebel and Ruttle (1936) described the ^{split} the ^

in Tradescantia taking place at metaphase, two mitotic cycles prior to their separation. Smith (1932) on Galtonia found the split occurring during the prophase of the division previous to separation.

Nucleoli

The size, number, and formation of nucleoli have been given prominence in recent cytological works. Digby (1910) reported that two small chromosomes were attached to the nucleolus in Galtonia, however, it has since been shown that a medium-lengthed pair is attached to the nucleolus (Smith, 1933). Lesley (1938) discovered that in tomato cells the nuclei with chromosomes that had satellites increased in size, had larger nucleoli than the cells with ordinary satellites. Pathak (1940) working with cereals, finds the nucleoli are organized in the telophase by a pair of chromosomes with secondary constrictions. The nucleoli usually fuse and the two chromosomes may be seen attached to the nucleolus. The nucleolus is organized around the thread of the secondary constriction. The number of nucleoli is proportional to the number of chromosomes with secondary constrictions. The tetraploid and hexaploid Triticum and Aegilops species have four and six nucleoli respectively.

Berger (1940) working with polysomatic cells in Spinacia, found that the diploid cells always have either two small or one large nucleolus. Tetraploid cells have four small nucleoli, one large, or an intermediate number in the process of fusion. Bhaduri (1939) found that the presence of unpaired nucleoli in Oenothera indicated heterozygosity of the species. Mensinkai (1940) from abnormalities found in the satellites of various species of Allium concluded that the satellite is only the rolled-up end of the chromosome-helix. Cave and Bradley (1943) found as many as six chromosomes associated with the nucleolus of Miersia chilensis at pachytene. The nucleolus forming regions are apparently incorporated in the short arms of certain rod chromosomes. "In this species no satellites have been found unless it may be considered that the short arms of certain rod chromosomes have become so reduced that they consist of nothing more than satellites and nucleolus forming regions." This lack of restriction of nucleolus formation has also been found in Trillium.

Warmke (1941) states that it is probably correct to consider the satellites as separated from the main body of the chromosome by a secondary constriction. These secondary constructions are linked with nucleolus formation.

The beginning of nucleolus formation at the end of anaphase was recorded in this paper. It was also noticed that all gametophyte cells had but one nucleolus, the sporophyte cells had either one large nucleolus or two small ones, and the endosperm cells all showed three small nucleoli.

Fragmentations and Structural Hybridity

Fragmentation is the transverse splitting of the chromosomes. It has been found by several workers that fragmentations may be caused by X-rays but that the broken ends tend to rejoin more readily at high temperatures. Sax and Enzmann (1939) found this occurring in Tradescantia microspores. Faberge⁽¹⁹⁴⁰⁾ also working with pollen grains in Tradescantia concluded that heat caused the broken ends to join up more readily. Darlington (1940) reported a transverse fragmentation of the centromere. The new telocentric chromosome so formed is sub-efficient; it reproduces and divides with less regularity than the complete chromosomes. He states that in the diploid fritillary it is a consequence of the non-co-orientation of bivalents as well as univalents, there must be a specific and exceptional capacity for misdivision or incapacity for correct division inherent either in the centromeres or in

the organization of meiosis. These telocentrics lag and often become extranuclear bodies. Misdivision is the only known means of adaptation in the size and functions of the centromere. The telocentric chromosome can restore the intercalary position of its centromere by a secondary change, the two sister chromatids becoming concurrent arms. The new chromosome with two identical arms may then be described as an isochromosome." Rhoades (1940) examining the telocentric chromosomes in maize found that they were unstable and formed isochromosomes, similar to those reported by Darlington. Bhaduri (1939) found that in Oenothera blandingi instead of the normal complement of $2n = 14$ chromosomes, some cells had 15 chromosomes. This has been ascertained to be due to real fragmentation. This fragment belongs to one of the long chromosomes to which it is normally attached by a secondary constriction. Levan and Emsweller⁽¹⁹³⁸⁾ studying Nothoscordum reported that early studies showed one fragrans form with $2n = 16$ chromosomes, all with median attachments. A closely related form had $2n = 18$ chromosomes, four of which had terminal attachments. Since these terminally attached chromosomes were about the same length as one arm of the longest bi-armed chromosomes

present, their origin has been explained by fragmentation of one long bi-armed chromosome in the eight chromosome genom. The new form discovered by these authors had nineteen somatic chromosomes, thirteen with median attachments and six with terminal.

A second type of structural hybridity was reported by Cave and Bradley (1943) resulting from the fusion of two chromosomes with terminal attachments to form a V chromosome. They account for the origin of these V chromosomes in one of two ways: "(a) reciprocal translocations between two non-homologous rod chromosomes and consequent loss of one centromere and both short arms, or (b) the fusion of the centromeres of two non-homologous rod chromosomes".

Evidence was found of changes in chromosome structures in the somatic cells ^{of} Galtonia similar to those reported above. The telocentric chromosome found in the root tip anaphase (Fig. 9) behaves in the same manner as those reported by Darlington. There was also some evidence found of the formation of median chromosomes from two terminally attached chromosomes. However both these types of abnormality were very uncommon, and no conclusion could be made as to their genetic significance in this form.

Chromosome Pairing

The pairing of homologous chromosomes in the somatic divisions has been reported by several workers, but the subject is still controversial.

In Galtonia candicans, somatic pairing at early metaphase and at anaphase has been reported by Newton (1924). He considered the metaphase pairing to be a loose arrangement and the anaphase association to be true pairing. The material studied in this paper showed no signs of any paired arrangement of the chromosomes in the root tips at any stage, but quite definite pairing of the chromosomes in the somatic cells of the ovule. The fact that the ovule mitoses resemble meiosis in several ways, namely - the pairing of chromosomes, the extreme contraction of the chromosomes, and the occasional presence of nucleoli at late prophase, would suggest that the conditions which bring about the meiotic divisions have some effect on the mitotic chromosomes. What these conditions are has not been determined, some workers suggest that they are caused by age, some suggest the presence of necrohormones (see Gustafsson (1939) who suggests that they are related to a sap intake and growth period, and Darlington (1936) who explains meiosis as the consequence of a shift in

time coordination between external and internal factors in the development of the chromosomes. The prophase begins before the chromosomes have divided, that is, it is precocious. All of these theories seem to suggest that the change from mitosis to meiosis is caused by some external, physiological condition about which very little is known. If it is physiological, I can see no reason why it cannot effect the cells surrounding the germ cells, to some extent. Gustafsson (1939) cites Fagerlind who found in his review of the cytology of Rubiaceae found in species with pronounced degeneration of the meiotic cells and nuclei, somatic cells can be changed in a meiotic direction, showing chromosome contraction and synzesis stages. This suggests that some substance is released by the disintegrating cells and that this substance affects adjacent cells. Gustafsson (1939) found that the intake of sap and growth of a nucleus changes from meiosis to mitosis. "If no growth and vacuolization period sets in division is reductional. After a certain amount of hydration and vacuolization division becomes equational, but is still meiotic in character, and only when growth and hydration have reached another threshold is division mitotic."

Bhaduri (1939) cites Marquardt as observing cases of apparent pairing of somatic chromosomes in the anther archesporium. Marquardt suggested that this may represent a step towards conjugation, which will take place in the following nuclear division. Bhaduri does not agree with this view.

Very definite pairing of somatic chromosomes is found in all the tissues of the Diptera (Metz, 1916). This has also been reported in some of the other insects. Since this condition is so constant in the tissues, it is unlikely that it is the same time of pairing as that found in the ovule cells of Galtonia.

Translocations and Fusions

Types of translocations and terminal fusions, both induced and those occurring in nature, have been studied by many cytologists. Probably the most thoroughly investigated phenomenon is the ring formation or catenation in Oenothera. Sturtevant and Dobzhansky (1934) drew a parallel between the pairings found in Drosophila and the catenations found in Oenothera. They found that the pairing was between like regions on the chromosomes and not the whole chromosome. The theory was advanced that translocations between non-homologous chromosomes caused ring formations

in Oenothera. They found cytological evidence for such translocations in Drosophila. Bhaduri (1939) agrees with the above explanation of the origin of these chains. Sikka (1940) gives a review of the work done on Oenothera. He further suggests that since the force which holds the chromosomes together is synaptic, and since, in a diploid, synapsis is between one maternal and one paternal chromosome, it follows that these chromosomes in the ring are alternately paternal and maternal in origin. Both Sikka and Jacob (1940) recognized that there is a particular arrangement of the chromosomes for each species or type. Mensinkai (1940) found rings and chains formed in Allium by the same type of reciprocal translocations between non-homologous chromosomes. A similar condition was reported in Primula Kewensis by Upcott (1940).

Somatic Bridges

The chromosome bridges formed at anaphase in some abnormal nuclei may be caused by several different conditions, namely - the formation of dicentric chromosomes, the lagging of acentric chromosomes, or by the adhesion of chromosomes. Upcott (1936) working with triploid tulips reports the first type of chromosome bridges. In all plants studied, dicentric chromatids are formed at

meiosis as a result of crossing over between relatively inverted segments of chromosomes pairing during prophases. These chromatids form bridges at first and second anaphases. "The stretching of the bridges at anaphase bears out the view of Belar and Darlington (1932) that the anaphase separation is determined by two agents, first, by the mutual repulsion of the centromeres, and later by the stretching of the central region of the spindle which completes the process by pushing the chromosomes farther toward the poles. Observations show that the spindle expansion takes place in two zones - between the equator and the poles." The region adjacent to the centromere is more susceptible to uncoiling. "The fact that the strain causing the uncoiling is not transmitted equally across the bridge but remains localized suggests that there is considerable friction between the chromosomes and the spindle material."

Mensinkai (1940) reports a somatic bridge in a root tip of Allium. He considers this bridge formation to be "due to some adverse external circumstances influencing the physiology of the particular nucleus producing denaturation of chromatin in the particular chromosome." He further says that the lagging of chromosomes and bridge formations in

somatic mitosis is not of significance unless in the germ track, when they affect the germ cells and induce transmissible variations.

The third type of somatic bridge is not caused by translocations but is merely adhesions of the chromosomes. White (1936) treated Orthoptera material with X-rays. He found that after irradiation the chromosomes appear to have become more viscous, so that they only separate with great difficulty. The two chromosomes adhere in spite of the repulsion between the spindle attachments which is pulling them apart. The result is that the two groups of anaphase chromosomes are held together by a "bridge". White suggests that this and other occurrences in irradiated material is probably due to a general disintegration of the chromosomes.

The bridges form in the ovule cells of Galtonia in this last manner. (Figs. 17 and 18) The chromosomes appear to have become viscous; they have lost their individual shape; they adhere instead of separating normally.

Sax (1938) also reported the clumping of the chromosomes as the first effect of irradiation. The treatment apparently affects the chromosome envelope. The fusions resulting from the clumping of chromosomes may also be produced by heat and age.

Chromatid Fragmentation

Sax (1938) stated that X-rays may break both chromatids while ultra-violet rays usually break only one of the two chromatids. According to this author, the chromosomes are in the form of relaxed coiled chromonemata during the resting stage. An X-ray hitting a chromosome can break two adjacent gyres and the reunion of broken ends in new associations will produce small deficiencies and inversions. The size of the deficiency or inversion will depend on the stage at which the chromosome was hit, since the size and number of the gyres vary at different stages. The same worker (Sax, 1941) has done further work on chromosome and chromatid fragmentation induced by X-rays in regard to dosages and time relations.

Ultra-violet rays from sunlight or changed temperature conditions might possibly have caused the single chromatid break shown in Figure 19 and the resulting loop formation.

Diplochromosomes and Polysomaty

I have grouped these two types of abnormality together because both of them seem to involve a split of the chromosome or chromatids and a delay or failure of division.

Diplochromosomes were first reported by White (1935). He found these V-shaped chromosomes present in approximately the diploid number. Each arm of the V is longitudinally split into two chromatids. Each chromosome is a compound structure with two genetically homologous arms and a spindle attachment in the middle. White says that diplochromosomes have eight chromatids. He apparently considers the arms on each side of the centromere as separate chromatids. Barber (1939) speaks of diplochromosomes as four chromatids passing through one centromere. These are formed by repeated division of the chromosomes without mitosis. "This may be described as progeny pairing, that is, it maintains the status quo in contrast to pachytene pairing which is an active type of pairing."

Reduplication of chromonemata during resting stages has been found quite frequently in both plants and animals. Bauer (1936) postulated that the large salivary gland chromosomes of Drosophila were composed of a large number of closely united chromonemata. Painter (1940) describes the salivary gland chromosomes as originally having four chromatids. After synapsis the compound chromosome grows in diameter and length. The growth in size of the chromomeres is presumably due to reduplication without a

visible division of parts. The uncoiling which partly causes the increase in length would also explain why we see a more complex pattern of bands. Berger (1938) found the cells of the larval ileum of Culex increasing in size but not in number. The increase in size is paralleled by an increase in chromosome number. According to Berger, "the nucleus retains the typical resting stage condition throughout the growth and chromosome multiplication period. Subsequent divisions show orderly somatic synapsis and are apparently somatic reduction divisions giving smaller cells with the reduced chromosome number".

Gentcheff and Gustafsson (1939) describe what they called "polysomatic cells" in Spinacia. They found that in most of these cells the chromosomes are closely associated in pairs. They also found that these cells were larger than those with a normal chromosome complement. Some nuclei as soon as the chromosomes show up in early prophase may have a quadripartite structure. Two major threads, each consisting of two chromatids, lie separate from each other except for a marked twisting. This twisting is probably identical with the relational coiling of chromatids in mitosis. Figure 7 shown by these workers looks very similar to Figures 21a and 21b of Galtonia.

From the structure and relations of the chromosomes I would assume that they originate in a similar manner.

Berger (1940) reinvestigated this condition in Spinacia and added that polyploid cells with unpaired chromosomes have undergone at least one mitotic division since the time of double reproduction because the chromosomes remain paired until the nucleus divides, at which time the paired relation is lost. Erwin (1939) reports a similar condition in the roots of Cucumis melo.

All workers agree that this reduplication of the chromosomes occurs during the stages when the chromosomes are non-mobile.

Conclusions and Suggestions for Further Research

The abnormalities found in the ovule cells of Galtonia probably resulted from a combination of factors such as: ultra-violet rays from sunlight, heat, age, and possibly hormones or physiological conditions which are ordinarily associated with meiosis. Whatever condition or conditions caused the aberrations in the somatic cells of the ovule apparently did not affect adversely meiosis or the development of the germ cells. Since the forces affecting the surrounding cells act in this direction the normal behaviour during meiosis is to be expected. The results found confirm this expectation. The seeds used for germination purposes were taken from the same plants and the resulting root tips showed normal mitotic divisions. The fact that the seeds germinated successfully even after a year and a half, producing normal root tips, would indicate quite definitely that none of these abnormalities are carried into the germ cells. The developmental stages of the female gametophyte are also seen to be quite normal.

There are several points touched on in this paper which suggest further investigation. It would be interesting to discover just when the abnormalities begin to occur and whether or not they are confined to the flowering parts.

It was noted during the investigation that the somatic cells in young ovules showed more typically mitotic divisions. The question of polysomaty might also be investigated further to ascertain if this were merely a chance occurrence or whether it were common in certain tissues. The chromosome behaviour at the time of syngamy has not been observed and the analysis of these stages in the life history should be significant.

Summary

1. There are sixteen somatic chromosomes and although complete individuality exists, it is convenient to divide them into three size groups: four large pairs, two medium pairs, and two small pairs. These were identified very markedly at diakinesis.

2. Normal mitotic stages are described.

3. The nucleoli are associated with a pair of medium-lengthed chromosomes with satellites.

4. A single instance of a telocentric chromosome is the only abnormality found in the root tips.

5. Aberrations in ovule cells include pairing, fusions, chromatid fragmentation, and polysomaty.

6. The conditions causing these aberrations do not affect germ cells adversely. It may be that the forces promoting meiosis and germ cell development are potent to the extent of having an appreciable effect upon the surrounding tissues thereby causing the aberrations present. in the direction of pairing, synapsis, and precocious splitting.

Explanation of Plates

All drawings were made with the aid of a camera lucida. The magnification at table level was about 4800 diameters. The drawings have been reduced one half in reproduction. All drawings are at the same magnification.

Plate I

- Fig. 1. Individuality of chromosomes at diakinesis.
- Fig. 2. Ring arrangement of pair No. 1.
- Fig. 3. Polar view of metaphase plate in root tip, showing individuality of the chromosomes and the random arrangement.
- Fig. 4. Polar view of metaphase plate in ovule cell mitosis, showing paired arrangement of the chromosomes.
- Fig. 5. Lateral view of metaphase in root tip mitosis.
- Fig. 6. Chromosomes at middle anaphase in root tips.
- Fig. 7. Telophase chromosomes in root tips showing spiral chromonemata.
- Fig. 8. Prophase chromosomes having two more or less chromonemata.

Plate II

- Fig. 9. Telocentric fragment in root tip telophase.
- Fig. 10. Prophase in ovule cell showing pairs of thread-like chromosomes.
- Fig. 11. Polar view of mitotic division in ovule cell, showing distinct meiotic tendencies of the chromosomes.

Fig. 12. Terminal fusions of mitotic chromosomes in ovules during the prophase.

Fig. 13. Terminal fusion of homologous chromosomes in ovule cells at metaphase.

Fig. 14. Two chromosomes attached to one spindle attachment at early anaphase in ovule cells.

Figs. 15, 16, 17. Fusions of non-homologous chromosomes at prophase and metaphase.

Figs. 18, 19. Somatic bridges at middle and late anaphase.

Fig. 20. Chromatid break forming a loop in a mitotic chromosome.

Figs. 21a, 21b. Two sections of the same nucleus showing polysomatic condition. The chromosomes are grouped in pairs.

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Plate I

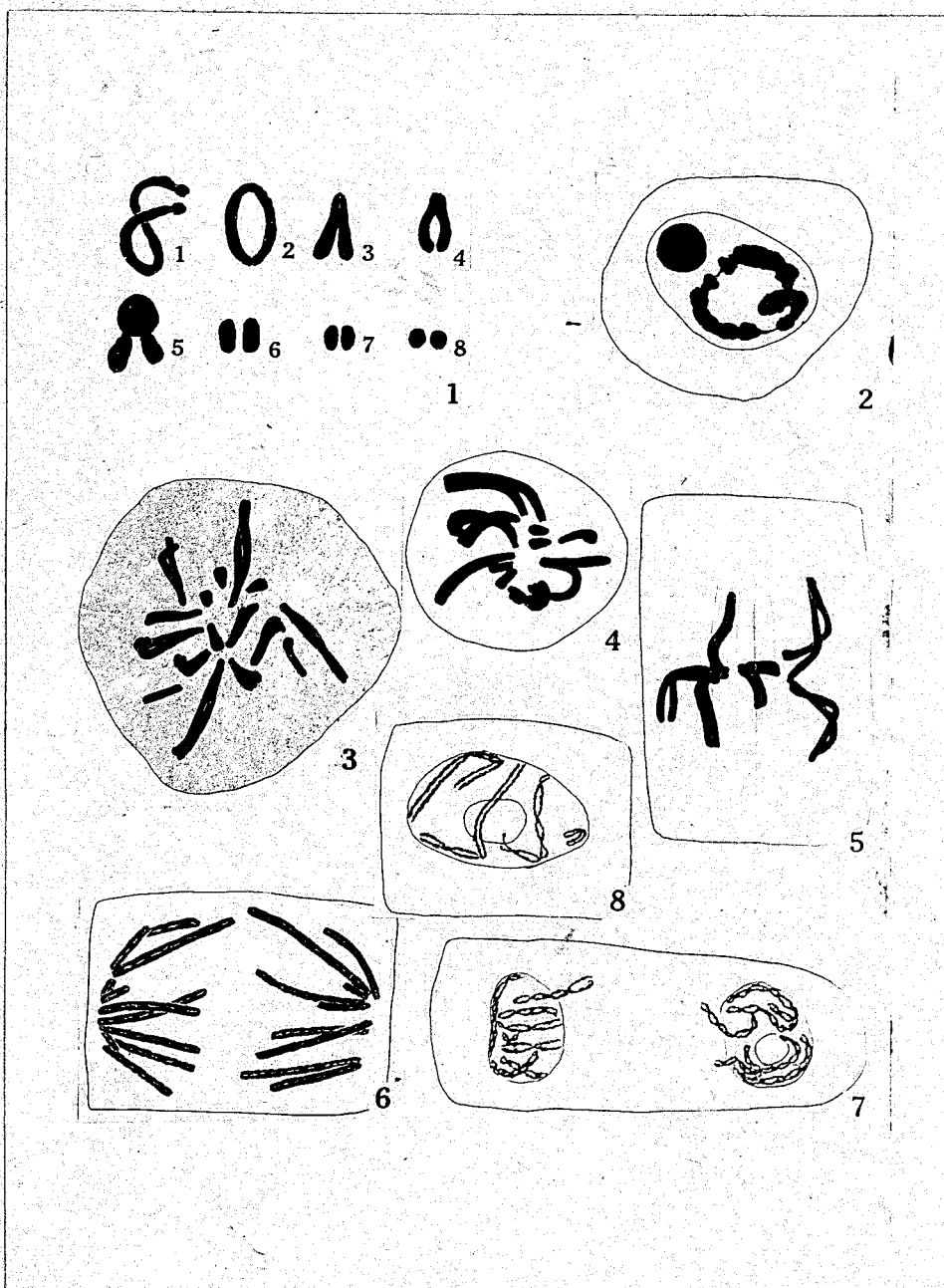


Plate II

