DIFFERENTIAL REACTIVITY

in the

CHROMOSOMES OF *TRILLIUM* SPECIES

by

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Introduction

The study of chromosome structure and behaviour antedates by several decades the establishment of genetics on a scientific basis. Beginning, inevitably, as a purely descriptive discipline, chromosome cytology has in recent years expanded and branched until it promises to play an important part in the integration of many fields of scientific investigation. Already it has invaded such subjects as molecular physics and protein chemistry, and made a start, however small, in the interpretation of genetical and embryological observations in chemical terms. With the improvement of present methods of investigation, and the development of new techniques, we may look hopefully for great advances along these lines.

The present investigation is concerned with the effects of environmental changes on the structure of the chromosomes of several species of Trillium. It is hoped that the result may be an addition, however small, to the knowledge of chromosome behaviour which is prerequisite for the complete understanding of the methods of inheritance.

Review of Literature

1. Differentiation Deduced from Theoretical Considerations.

Even before the chromosome theory of heredity was placed on a firm foundation, when it was, in fact, a subject of pure speculation, several workers proposed on theoretical grounds a longitudinal differentiation of the chromosomes. Roux, in 1883, suggested that the hereditary "qualities" were arranged in a linear series, and later Weismann (1892) proposed the theory that the idants, or chromosomes, contained a linear series of ids, which were themselves composed of smaller units -- determinants and biophores.

The establishment of the chromosomes as the material basis of heredity inevitably implied some differentiation within them. The "factors" of Mendel (1865), called "genes" by Johanssen (1909), must be on the chromosomes, and therefore the chromosomes can not be structurally uniform. The linear order of the genes, suggested previously by several workers, was finally given experimental support by Sturtevant's (1913) observations on double crossing-over and interference, which resulted in the first chromosome maps. In broad outline this conception of chromosome structure is held by most workers in the field to-day, with the notable exception of Goldschmidt. He discarded the theory of the individuality of the genes

(1940) and claimed that the whole chromosome was the unit in heredity. This theory however, like the gene theory, implies submicroscopic differences in structure along the length of the chromosome, since even minute rearrangements of the chromatin result in phenotypic changes. In fact, the two theories seem to be basically very similar.

This differentiation, whether genic or not, is below the limits of microscopic resolution. It must, therefore, be studied by indirect methods, among which is the observation of various types of visible differentiation.

2. Morphological Differentiation.

Metzner, as early as 1894, described certain parts of the chromosomes which were a constant feature, and were morphologically distinguishable from the main body of the chromosome. He called these structures "Leitkorperschen" because of their apparent role in controlling chromosome movement. Since Metzner many names have been given to these structures, but the two in most common usage are "kinetochore" (Sharp, 1934), which like Metzner's term implies function, and "centromere" (Darlington), a name based on purely morphological considerations.

The structure of the centromere has been the subject of considerable discussion in recent years. It was described by Belar (1929) as having the appearance of a clear spot in living chromosomes. Lorbeer (1934) considered

the centromere to be like any other chromomere, except in its specialised function, but most recent workers have described it as a compound structure, as suggested by Nebel (1935). Schrader (1936,1939) described the kinetochore as consisting of a spindle spherule embedded in a cup-shaped commisural region. He found that it had different staining reactions from those of the rest of the chromosome, in that it reacted to several mitochondrial stains. Functional fragments were thought to be formed if the kinetochore were broken before its metaphase form had been reached. Darlington (1939) deduced from observations of misdivisions of the centromere that the normal method of division was by "explosion" in a fluid. He therefore suggested that structurally the centromere contained fibrous determinants within a fluid medium. Nebel (1939), in a review paper, suggested a three-partite structure for the kinetochore. The central achromatic body (= commisural region of Schrader) contained chromatic kinetic bodies (= spindle spherules); in addition to these were special chromomeres connecting the achromatic body with the chromosome arms. More recently (1941) Matsuura has suggested a structure for the kinetochore very similar to that of the rest of the chromosome. He considered the kinetonema, part of and continuous with the rest of the chromonema, to be embedded in a matrix which was fully developed by metaphase. The behaviour of the kinetonema and matrix were thought to be

similar to the rest of the chromosome, but relatively retarded, and with no coiling of the kinetonema.

In addition to the centromere (= primary constriction) which is an integral part of all functional chromosomes, secondary constrictions have frequently been reported. S. Nawaschin (1912) first recorded satellites in Galtonia, and since that time they have been seen by many workers. Heitz (1931a,b), basing his conclusions on the results of observations on a number of different plants, explained the relationship of satellites to nucleoli. McClintock (1934) found that in Zea mays another part of the chromosome, and not the constriction itself, was concerned with the organisation of the nucleolus. Not all organisms show definite constrictions near the nucleolus organising region. In Trillium kantschaticum Pall., for example, Matsuura (1935) found the nucleolus attached to the end of the a-chromosome (the E chromosome in our nomenclature, following Huskins and Smith, 1935). Chromosome pairs differing in size of trabant have been reported by Medwedewa (1929) in Crepis discoridis and by Lesley and Lesley (1935) in Lycopersicum. Medwedewa reported greater vigour in plants with the larger satellites, but Lesley and Lesley found no difference in the tomato between plants with large and with small satellites, from which they concluded that the satellite was genetically inert.

Large numbers of secondary constrictions, not all

concerned with nucleolus formation, have been reported in a number of organisms. Frequently, however, special fixatives were necessary to render these constrictions visible. The best results were usually obtained with fixatives containing little or no acetic acid, which causes swelling of the chromosomes with consequent obliteration of the constrictions. Geitler (1929) observed "chromomeres" (the result of many constrictions) in the somatic metaphase chromosomes of Crepis capillaris, and his observations were confirmed by Kakhidze (1939), who found the chromosomes to be uniformly divided into "chromomeres". Ellenhorn (1937) reported "chromomeres" in the mitotic chromosomes of Allium cepa, while Schmargon (1938) reported a similar appearance of the chromosomes in Crepis, Allium and Secale cereale . In arctic species of Ranunculus and grasses Flovik (1936,1938) found many secondary constrictions, nearly three per chromosome in one Phippsia species.

In the study of chromosome structure and behaviour one of the phenomena investigated most extensively has been the coiling of chromonemata. The greater part of the work has been done on meiotic chromosomes in attempts to gain evidence on chiasma formation, etc., and also because the internal structure of the chromosomes may be seen much more readily in meiotic than in mitotic chromosomes. The main theories concerning chromonema coiling have been presented and discussed by Darlington (1935,1937, p.483 et seq.),

Kaufmann (1936), Huskins (1937), Nebel (1939), Wilson and Huskins (1939), Kuwada and Nakamura (1940) and Sparrow, Huskins and Wilson (1941). The theories of coiling may be divided roughly into those which stress the importance of forces within the chromonemata in the formation of the spirals, and those which stress the interaction of chromonema and matrix. The relative importance of these factors is of interest in the interpretation of the structure and origin of "differentially reactive" regions.

Spiral structure in mitotic chromosomes has been observed, among others, by Sax (1936), Upcott (1936), Geitler (1938), Coleman (1940). Sparrow (1942), in describing the structure and development of the chromosome spirals in the microspores of Trillium, stated that "... the chromonemata in each metaphase chromatid and each anaphase chromosome form a largegyred, hollow spiral. This spiral develops gradually during prophase by an increase in gyre diameter and a decrease in gyre number and in chromatid length."

3. Heterochromatin and the Nucleic Acid Cycle.

Heterochromatin. Although Rosenberg, as early as 1904, had drawn attention to the apparent coincidence between chromosome number and the number of chromatic bodies on the surface of the resting nuclei of some plants, the study of the differential behaviour of certain chromosomes and

chromosome parts owes its origin mainly to the work of Heitz (1928,1935). He described two types of chromosome material, euchromatin and heterochromatin. The former showed the usual cyclic changes through mitosis, with greatest condensation at metaphase and anaphase. The latter differed from it by retaining its individuality at telophase, and staining deeply at prophase (this type of behaviour has been referred to as heteropycnosis). The Feulgen nuclear reaction did not distinguish between the two types of chromatin, but aceto-carmine gave good differentiation. Probably similar to this are the chromatic knobs which have been observed in the meiotic prophase chromosomes of Zea mays. Longley (1938) found as many as 18 in some strains of corn; a possible relationship between these and the supernumerary B chromosomes may exist, since Longley found the latter most common in strains with less than seven prophase knobs.

Chromatic bodies just within the membrane of the resting nucleus have been reported in many plants. Grégoire (1931) called these bodies "euchromocentres", , but the terms in more common usage are chromocentre and prochromosome. These chromocentres appear to be similar to the heterochomatin reported by Heitz. Both Doutreligne (1933), who studied a number of plants, and Manton (1935), who studied polyploids in the Cruciferae, reported that the chromocentres corresponded in number and position with the chromo-

somes, and agreed with Smith (1934) that they represented those parts of the chromosomes adjacent to the centromeres. These parts remained condensed through the resting stage, and in "sister" prophases they appeared in the same positions they assumed in the previous telophase. Kuhn (1929), however, believed that the chromocentres bore no relationship to the chromosomes of the following prophase, but dissolved in the karyoplasm before each mitosis. Marquardt (1937) in Oenothera and Coleman (1941) in Rhoeo related the chromocentres to the differential segments of the chromosomes. Because of this condensed condition these parts are therefore prevented from crossing-over. Contrary to Smith, who found no fusion of the chromocentres in Impatiens in either somatic or premeiotic nuclei, Coleman found that in Rhoeo they congregated into one or two masses at pachytene.

Several types of heteropycnosis have been reported other than that described by Heitz. Patau (1937) found that the ends of the chromosomes of Collozoum exhibited what he described as "Pseudoheterochromatie". In telophase they appeared more condensed than the other parts of the chromosomes as a result of the relaxation of the chromatid spirals being retarded. This overcondensed condition, however, lasted only up to the resting stage, and not through to the following prophase. In the early spermatogonial divisions of Locusta migratoria, White (1935) reported a phenomenon which he called "negative heteropycnosis". Throughout the

division, from prophase to anaphase, the X chromosome was less contracted than the antosomes. It remained long and thin, with an irregular outline. This effect was less marked in the later divisions, and in the first meiotic prophase the X chromosome showed "positive heteropycnosis", having reached the metaphase degree of contraction long before the autosomes. In a later paper (1940) White reported a more detailed study of both positive and negative heteropycnosis in a number of genera of grasshoppers. In the Tettigonidae only positive heteropycnosis of the X chromosomes was found, and that only at prophase. The degree of contraction of the X and autosomes was the same at metaphase in all spermatogonial divisions. In the Acrididae, on the other hand, both negative and positive heteropycnosis were found. According to some reports the undercondensed X does not stain as deeply as the autosomes, but White thought that this might simply be an optical illusion caused by the irregular outline of the former. The ends of the X chromosomes frequently appeared more contracted than the interstitial parts. In meiosis the X exhibited positive heteropycnosis. Pear-shaped from leptotene to pachytene, it assumed a sausage shape in diakinesis and metaphase, with a smooth outline and a slightly greater diameter than the antosomes. The length of the X chromosome was found to be the same at diakinesis, when it showed positive heteropycnosis, as at early

spermatogonial metaphase, when it was negatively heteropycnotic.

Heterochromatin frequently appears to be non-specific in its associations, the best known evidence of this being the formation of a single chromocentre in the salivary gland nuclei of Drosophila. According to Bauer (1936) this chromocentre is entirely chromosomal in nature and not an apposition of extraneous material. The strong attraction between these parts is further seen in the findings of Masing and Sokolow (1937). These workers reported a translocation from the right arm of chromosome II to the chromocentre (probably in the same chromosome) in D. melanogaster. In the salivary glands the extreme attraction of the heterochromatic parts caused the translocated segment to form a loop. Schrader (1941) found heterochromatic parts of non-homologous chromosomes paired in one of the Hemiptera. The sex-chromosomes and the ends of the autosomes are heterochromatic in Edessa. Schrader observed a "bouquet" at synapsis in which the heterochromatic parts formed a peripheral aggregation. He found that at late pachytene this aggregate broke up into smaller ones which might contain both ends of one chromosome, or one end of each of two different chromosomes. He attributed this non-homologous association to simple cohesion of the heterochromatin.

From the cytological evidence Heitz suggested the

possible genetical significance of heterochromatin. At first, since the sex-chromosomes were most frequently heteropycnotic, he thought it was directly related to sex, but later he changed his mind on this point. The genes are thought to act in the "resting" nucleus, when the chromosomes are passive. The telophase changes in the chromosomes, therefore, suggested to Heitz that heterochromatin was genetically inert, since heterochromatic parts remained in the typical condensed condition of kinetic chromosomes. He suggested two possible relationships between heterochromatin and euchromatin (1929,1932). The heterochromatin might either be passive, its genes inactivated, or it might contain no genes.

Most workers agree with Heitz that heterochromatin is less active, genetically, than eu-chromatin, but they are not agreed on its structure, or whether the two types of chromatin are fundamentally different. This disagreement may result in part from the use of different material. Thus, Muller and Gershenson (1935), considering the heterochromatic regions in Drosophila, suggested that these do not contain a coiled chromonema with degenerate genes, but are essentially non-genic material derived from a few active genes specialised for chromatin production. In support of this hypothesis they stated that X-ray breaks usually occurred at definite positions in the heterochromatin, so it must be made up of relatively large blocks. Muller and Prokofjeva

(1935) found that the inert region of the X chromosome was faintly striated, like euchromatin. They also reported that it was approximately the same length in mitotic metaphase as in the salivary chromosomes. They considered this to be evidence that it never coils, but merely forms an accretion of chromotin, and suggested that this might explain its inertness -- the chromonema was not truly inert, but merely encrusted.

White (1940), however, found that in the grasshopper the heterochromatic parts were spiralled. Clear relic coils could be seen in the X chromosomes both at resting stage and prophase, whether they exhibited positive or negative heteropycnosis. He believed that at metaphase the negatively hetropycnotic X chromosomes had more gyres per unit length than the autosomes, but differed from the latter by having thinner chromonemata. That heterochromatin is not fundamentally different from euchromatin was indicated, White thought, by the fact that a given region might be heterochromatic at one time and not at another. For example, the X chromosome is heteropycnotic in the male but not in the female of Saltatoria (Orthoptera), thus allowing crossing-over in the X's of the female.

Inert regions are not always heterochromatic, but may sometimes be structurally indistinguishable from active ones. For example, in Drosophila ananassae Kikkawa (1937) found that there were four pairs of long chromosomes

(X - 3A) with submedian centromeres, but only three linkage groups, which suggested that one pair of autosomes was inert. Yet in ordinary mitoses the chromosomes all appeared structurally similar. In the salivary gland nuclei of female larvae he found six long chromosome arms, and one tiny pair of strands embedded in the chromocentre. Since a haplo-IV fly was found, lacking one of the smallest pair of autosomes, Kikkawa assumed this to be the inert chromosome. There was some evidence that it was partly homologous to the short arm of the Y chromosome.

Differential Reactivity. As was stated by White (1940), the environment may play an important part in causing visible differentiation of chromosomes or parts of chromosomes. In the organisms which he studied the internal environment, determined by the genotype, was the important factor in certain cases. In other organisms, however, the external environment may determine whether or not two segments of chromatin will be optically similar. That is, two segments may react in different ways to a new environment. This phenomenon has been called "differential reactivity" by Darlington and LaCour (1938).

Kagawa (1929) and Ellenhorn (1935) observed such a differential reaction of the root-tip chromosomes of Triticum to chloral hydrate. The roots were soaked in a weak solution of chloral hydrate for as long as one hour,

washed well and kept moist for several hours before fixation. After this treatment certain specifically localised secondary constrictions, which were not normally visible, could be seen in the chromosomes. Haga (1934) found a similar reaction to chloral hydrate in the somatic chromosomes of two species of Trillium, T. smallii and T. Tschonskii. Most of the constrictions observed were near the centromeres of the A,B,C and E chromosomes (Huskins and Smith's (1935) nomenclature), but one was found near the end of the E chromosome of T. smallii. It seems probable that this reaction is similar to the differential reactivity observed in this present investigation. Unfortunately Haga included no photographs by which this might be determined.

The environment which has most frequently caused differentiation in the chromosomes is low temperature. Thus, Delaunay (1929) observed structural differentiation of chromosomes more clearly after cold treatment, as also did Shmargon (1938) in Secale cereale chromosomes after 24 hours at 0°C. Kakhidze observed "secondary articulations" in the root-tip chromosomes of Crepis after precooling. Regions of reduced diameter and staining capacity have been observed, after cold treatment, in the chromosomes of a variety of organisms (Paris, Trillium and Fritillaria -- Darlington and LaCour, 1938, 1940, 1941; Trillium and Secale -- the present investigation; Triton -- Callan, 1942; Triturus --

Smith and Entin, unpublished). A similar type of differentiation was also observed in Trillium root-tip chromosomes by Coleman (1940) and in the pollen-grain by Sparrow, Huskins and Wilson (1941) with no special pretreatment.

In Paris polyphylla Darlington and LaCour (1938, 1940) found that after 2 days at 0°C. the ends of the long chromosome arms in root-tip mitoses were reduced to about half their normal diameter and stained less intensely than the rest of the chromosome. These special segments were constant in size and position, and could be seen from prophase to anaphase. According to these authors the differentiation was caused by a reduction in the thickness of the chromonema and the diameter of the helix in the differential segments. Concerning the length of the segments, they stated (1938) " ... The chromosomes are the same length when unstretched, whether they show the differential reactivity or not", and " ... the lengths of the differential reactive segments are the same when showing their special reaction as in other preparations similarly treated when not showing it." In 1940 they also stated " ... Comparison of normal and treated material --- shows --- a reduction in staining capacity and diameter in the differential segment of the chromatid, without a change in its length.

Differential reactivity was also studied by Darlington and LaCour (1940) in the mitotic chromosomes of five species of Trillium (T.sessile, T. recurvatum, T. stylosum, T. grandiflorum and T. erectum). The differential segments which they found after 5 days at 0°C. were similar to those reported in Paris, but in Trillium they were not all at the ends of the arms. They found a high degree of variability in the size and position of the differential regions both between and within plants. In the case of T. grandiflorum plants, however, part of the variation appears to have been the result of a mistake in classification. Plants 3 to 7 were most probably T. stylosum. In four of the five species one pair of chromosomes never showed any differentiation. Of the remaining 16 pairs of chromosomes 14 showed "hybridity". That is, homologues differed in the size, or by the presence and absence of a differential region. Darlington and LaCour claimed that this was evidence of genetic (structural) hybridity which must have arisen by loss and translocation. It would be of interest, in connection with this interpretation of structural hybridity, to know how many nuclei of each plant were examined. On the basis of the apparent loss of certain differential regions from individual plants they concluded that these segments must be genetically inert.

From observations of anaphase figures in T.

stylosum and T. grandiflorum (?) Darlington and LaCour discovered a mechanism which they believed might have brought about this high degree of hybridity. The differential regions frequently remained associated during the anaphase separation of the chromosomes. One or both of the paired segments sometimes became much attenuated, and might break. In this way deficiencies or duplications of all or part of a differential region might occur. Although this mechanism might account for differences between terminal differential regions it is difficult to conceive how it could account for differences between interstitial ones.

Darkly staining chromocentres are present in the resting nuclei of both Paris and Trillium. In P. polyphylla nuclei Darlington and LaCour reported 13 chromocentres, which they identified with the 13 differential segments in the metaphase chromosomes. In T. erectum, when 24 differentials were seen, the number of chromocentres was smaller, and not always accurately ascertainable. In T. sessile and T. grandiflorum they found 4 chromocentres per nucleus. They explained these three cases by assuming that chromocentres for small differential segments would not be observed. In the light of this series of observations, Darlington and LaCour concluded that the differential regions were truly heterochromatic, that is, they were over-condensed through the resting stage, and at

low temperatures were undercondensed at metaphase and anaphase.

Out of 21 species of Fritillaria which they examined, Darlington and LaCour (1941) found only one, F. pudica, which showed differential reactivity. As in the case of Trillium and Paris, they stated that a minimum of three days cold treatment was required for the root-tip metaphase chromosomes to become differentiated. Thirty-five differential regions and 23 chromocentres were observed. In none of the plants studied by Darlington and LaCour did they find differentiation during meiosis, and in the pollen grain only in Fritillaria.

The phenomenon of differential reactivity was observed by Callan (1942) in three species of the newt Triton. A minimum of 3 days exposure to cold was required to produce the differentiation. In Triton, unlike the plants used by Darlington and LaCour, both mitotic and meiotic chromosomes showed differentiation. Callan observed as many as 74 differential segments in the mitotic and 40 in the meiotic metaphase. These were all short segments, the majority close to the centromere, and contained in mitosis a narrower chromonema spiral than the other parts of the chromosome. He identified these segments with the chromocentres of the resting nucleus, and stated: " ... only those (nuclei) which have developed right through from the preceding resting stage in the cold

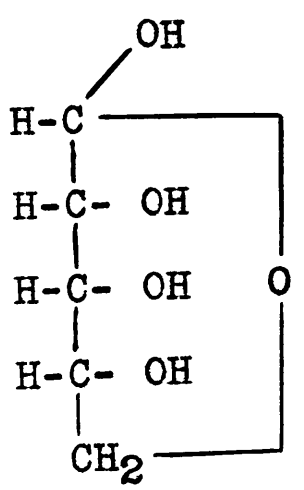
give the complete expression. Cells which were in prophase when the treatment started show ill-defined heterochromatic segments. These are visible as slight constrictions, but they stain almost as intensely as euchromatin." In meiotic chromosomes Callan found the differential segments to be completely Feulgen-negative following a diffuse pachytene stage which contained no chromocentres.

Both Darlington and LaCour, and Callan have interpreted differential reactivity in terms of nucleic acid.

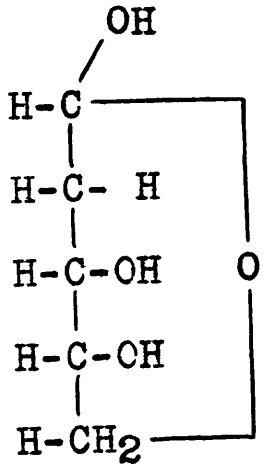
Chromosome Chemistry. Before one can understand the differences in structure and behaviour of eu - and heterochromatin and their significance, a comprehensive study of chromosome, and indeed cell chemistry is necessary. A great deal of work has been done on this subject, and present knowledge is briefly presented in such review papers as those of Shulman (1938) and Gulick (1941).

The first chemical analysis of nuclei was performed by Miescher in 1869, and published two years later (1871). He discovered in the nucleus of pus cells a substance which he called nuclein (= nucleoprotein). Other nuclear substancesdiscovered by Miescher were a sulphur-rich compound in mammalian nuclei, and protamine as a salt with nuclein (here nucleic acid) in the ripe sperm of salmon. Kossel (1884) discovered histone, a basic protein, also in a salt-like combination with nucleic acid.

For information on the structure of nucleic acids we are indebted in large measure to the work of Levene and his co-workers (see, for example, Levene and Bass, 1931). According to these workers the building blocks of nucleic acid, that is the mononucleotides, are composed of three parts, phosphoric acid, a pentose sugar and a purine or pyrimidine base, linked respectively by an ester and a glucoside bond. The nucleic acid unit is a tetranucleotide composed of four such mononucleotides linked through the phosphoric acid groups. Two types of nucleic acid have been identified, one of which contains the pentose d-ribose, and the other d-2-ribodesose (desoxyribose).



d-ribose



desoxyribose

The two types of nucleic acid can be distinguished optically by means of the Feulgen reaction. After mild hydrolysis a red colour reaction with leuco-basic fuchsin is given by thymonucleic acid, but not by ribonucleic acid. It is commonly believed that the hydrolysis splits purine bases off the thymonucleic acid, thus freeing aldehyde groups

which react with the modified Schiff's reagent.

It has already been stated that the early workers found the nucleic acid in combination with proteins. Mirsky and Pollister (1942-1943) have investigated the structure of these compound nucleoproteins. They found that those from different tissues were not identical. Wrinch (see for example 1939, 1941) has proposed a folded protein structure which would result in closed "envelopes" - a peptide fabric rather than polypeptide chain. This structure was proposed to account for the more or less constant molecular weights and specific properties of certain proteins. At the proper pH the proteins of the chromosome would attract to themselves rings of nucleic acid molecules.

Three principal methods have been employed for determining the distribution of proteins and nucleic acids in the cell. These are differential staining, digestion and the absorption of ultra-violet light. Perhaps the most useful of the staining methods is the Feulgen technique, which under controlled conditions is a specific stain for thymonucleic acid. Investigations have been made using a combination of these methods. For example, Mazia and Jaeger (1939 a,b) and Mazia (1941) studied the structure of Drosophila salivary chromosomes by both enzyme digestion and staining. After pepsin treatment the chromosomes were

shrunken, but stainable with Feulgen's. Conversely, after treatment with a nuclease they would not stain with either Feulgen's or acetocarmine, but the cytoplasm stained intensely. The chromosomes stained with ninhydrin (protein reagent). From these experiments they concluded " ... (a) that chromosomes contain a continuous protein framework, (b) that the architectural proteins may be protamines or histones, although an alternative interpretation is possible, and (c) that the structural integrity of the chromosome is independent of the presence of intact nucleic acid molecules". The alternative in (b) is that " ... nucleoproteins form a continuous framework through the chromosome, ...".

Caspersson (1937) also used enzyme digestion, but in combination with ultra-violet absorption in place of staining. The use of ultra-violet light for the detection of nucleic acids depends on the fact that any molecules containing pyrimidine groups show a powerful absorption band at about 2600Å. This method has the advantage over chemical ones, that it can be used to detect as little as 10^{-11} mg. of nucleic acid. Caspersson used trypsin to digest the proteins in the salivary chromosomes, and precipitated the nucleic acid as the lanthanum salt. The chromosomes fell apart into discs of nucleic acid.

Caspersson and his co-workers have used the ultra-

violet absorption method for an extensive series of investigations of the distribution of nucleic acids and various types of proteins in the cell. It was found (Caspersson,1937) that nucleic acid was localised in the chromosomes at all mitotic stages. In the metaphase chromosomes it was intimately mixed with protein. The type of protein was found to differ in various parts of the locust chromosomes (Caspersson, 1940 a), and thymonucleic acid and histone-type proteins were present in approximately equal amounts (Caspersson, 1940 b).

The thymonucleic acid content of the chromosomes was seen to increase during the prophase of meiosis (Caspersson, 1939 a, b) but the total nucleic acid content of the cell was constant through all later stages. On the basis of this evidence, and the observation of Caspersson and Schultz (1939) that the cytoplasm of growing cells contained a high concentration of nucleic acid not found in mature cells, Caspersson concluded that nucleic acid (probably a certain minimum amount) was necessary for cell division. The fact that the nucleic acid content of the chromosomes of Gomphocerus increased greatly from leptotene to zygotene, during which time it was not found in the cytoplasm or surrounding fluids, suggested that it must actually be synthesised at that time. It was concluded that chromosome contraction was not a function of nucleic acid synthesis.

A series of papers has been written on the chemical

structure and function of heterochromatin, particularly as it affects the nucleolus (Caspersson and Schultz, 1938,1940; Schultz and Caspersson, 1939; Schultz, Caspersson and Aquilonius, 1940; Schultz, 1941). The greater part of this study has been concerned with the phenomenon of variegation in Drosophila. Certain instances are known where translocations have brought genes from euchromatic parts of the chromosomes to the vicinity of heterochromatin. These genes then showed a variegation in their phenotypic expression. Cytological examination showed that an increase of nucleic acid had occurred in those bands of the salivary chromosomes near to the heterochromatin. This increase was greatest in the bands nearest to the heterochromatin and the genes nearest to the breakage point showed the greatest variegation in their expression. An extra Y chromosome reduced the effects, and in such cases more nucleic acid was found in the cytoplasm. According to Schultz and Caspersson (1939) "Since the accumulation of thymonucleic acid is apparently a local process at each chromomere, presumably it is performed by all genes. On such a basis the apparent inert character of the heterochromatic regions may be due to their specialisation in nucleic acid synthesis." The properties of heterochromatin were thought to include the capacity to form large amounts of thymonucleic acid, to effect the characteristics of regions translocated next to them and to affect the content of ribonucleic acid in the cytoplasm.

Interpretation. On the basis of this present knowledge of chromosome chemistry several workers have advanced chemical interpretations of the difference between euchromatin and heterochromatin. White (1940) reported Caspersson and Schultz (1938) to the effect that " ... the protein framework of the chromosome is responsible for the synthesis of nucleic acid." He therefore concluded that " ... a negatively heteropycnotic chromosome is one in which the protein framework has synthesised less nucleic acid than is usually the case, while a positively heteropycnotic one has formed more nucleic acid. It is probable that a certain minimum quantity of nucleic acid must be present before the chromosome assumes a smooth outline." That is, in negatively heteropycnotic chromosomes the chromonema was thought to be very little more than the protein framework, with insufficient nucleic acid to bring successive gyres into contact, while in positively heteropycnotic ones the great abundance of nucleic acid resulted in a compact, solid coil.

Darlington and LaCour (1940,1941) have interpreted differential reactivity in terms of the nucleic acid cycle and a limited supply of nucleic acid in the cell. They believed that at normal temperatures the differential segments contained an excess of nucleic acid in the resting stage, but the usual amount by metaphase, as do the B chromosomes of Zea mays. At low temperatures, however, the chromosomes were undercharged with nucleic acid at

metaphase. They claimed that to obtain this effect the cell must have passed through most of its resting stage at the low temperature, that is, the temperature had its effect before the chromosomes started to take up nucleic acid at prophase. They suggested that heterochromatin had a lower reactivity with nucleic acid than euchromatin (but compare Schultz and Caspersson, 1939), that is, the heterochromatin was chemically subordinate, as it was genetically. The low temperature reduced the supply of nucleic acid, and therefore at metaphase, when the demand was highest, the heterochromatic regions were short of it. The reason why some plants showed "allocycly" at low temperature and others did not was explained by assuming a higher requirement of nucleic acid in some, so that the supply could more easily be reduced below the minimum for all chromatin to get sufficient. Since the chromonemata were spiralled in the undercharged regions, they concluded that " ... nucleic acid seems to have no necessary relation to spiralization."

Callan (1942) agreed with Darlington and LaCour's interpretation of differential reactivity for mitosis in Triton, but found the situation slightly altered in meiosis. In the meiotic metaphase chromosomes the differential regions contained, according to Callan, thin non-staining strands the thickness of the leptotene threads, which contained no nucleic acid and were completely uncoiled. He explained the lack of coiling as the result of the absence

of nucleic acid, and compared this with the case in Orthoptera where he assumed that the nucleic acid was lost after spiralisation had occurred. With regard to this lack of coiling, however, we can quote Callan to the effect that " ... the segments are so short that in unstretched bivalents they are frequently obscured by nearby coils of the euchromatin spiral, and such segments resemble the constrictions between successive gyres." It seems probable, therefore, that the differential segments actually form a coil at neiosis, but were stretched in the bivalents which Callan observed.

Material and Methods

All four species of *Trillium* native to Quebec Province have been used in the present investigation. The plants were collected on or near the Island of Montreal, with the exception of those of *T. undulatum*, which were collected in the Eastern Townships of Quebec and in the Laurentians. The rhizomes of *T. erectum* and *T. grandiflorum* were collected at Ile Perrot, Ile aux Vaches and Montreal West, and those of *T. undulatum* at Lennosville and Piedmont. The *T. cernuum* plants, which had been collected near Montreal North, were kindly supplied by the Montreal Botanical Garden.

The plants were kept in flats or beds in the greenhouse until used. Root tip mitoses were studied in all cases, with the exception of certain counts of the number of chromatic bodies in the resting nucleus, which were made in anther wall and pollen grains. Unless otherwise stated all observations were made at metaphase. The fact that *Trillium* plants usually produce few new roots at one time has greatly hindered the investigation. Treatment with two root-promoting substances, indolyl butyric acid and a-naphthyl acetic acid, proved of no avail.

For most experiments cold treatment was provided by a commercial electric refrigerator which maintained a fairly constant temperature of 3°C. Constant temperature

chambers were used for temperatures other than 3°C., with the exception of "recovery" at 35°C., for which an electric oven was used.

Treatments other than temperature may be grouped under two headings: (a) physical, which included intense light and changes in pressure and moisture; (b) chemical, which included changes of pH, and the use of ether, a-naphthyl acetic acid, indolyl butyric acid, chloral hydrate, acenaphthene and colchicine. The method of treatment in each case will be described with the observations.

In order to obtain as uniform results as possible the same method of fixing, staining and mounting was used in all cases. Root tips were fixed for half an hour in a mixture of 3 parts absolute alcohol and 1 part glacial acetic acid, mascerated in 45% acetic acid at 60°C. for 20 minutes, and squashed and stained on the slide with aceto-carmine. The preparations were made permanent by draining off the aceto-carmine and replacing it directly with a modification of Zirkle's (1940) mounting medium. The formula used was:-

Venetian turpentine - - - - - - - - -	15 cc.
Phenol - - - - - - - - - - - - - - -	30 cc.
Propionic acid - - - - - - - - - - -	35 cc.
Acetic acid (glacial) - - - - - - -	5 cc.
Water - - - - - - - - - - - - - - -	20 cc.

Observations were made chiefly with a Zeiss 1.5 mm, 1.3 N.A. objective and 7x oculars, which gave a magnification of ca. 840x. The lens combination used for photomicrographs was a Zeiss 3 mm, 1.4 N.A. objective and a 7x ocular. Measurements were made from camera lucida drawings at 5000x magnification, for which the 1.5 mm, 1.3 N.A. objective and a 20x ocular were used. The occuracy of the measurements was dependent on the degree to which the chromosomes could be flattened, and was greatest, therefore, when they were most highly contracted.

Observations

1. The Appearance of Differential Regions.

When *Trillium* plants were kept at low temperatures certain segments of the somatic chromosomes became visibly differentiated from the remainder. Following Darlington and LaCour's (1938) nomenclature we have referred to the peculiar behaviour of these segments as "differential reactivity" and to the segments themselves as "differential regions". Differential regions may be seen, after cold treatment, from late prophase to telophase. No observations were made on living cells; however, staining was not found to be necessary in order to recognise the differential regions. In material which after 96 hours at 3°C. was fixed in 3:1 alcohol-acetic acid they could be seen as somewhat narrower segments of the chromosomes. Their structure also differed from that of the rest of the chromosome in that the alveolar appearance produced by this fixative was less pronounced. When the chromosomes were stained either with aceto-carmine or by the Feulgen technique the differential regions appeared understained relative to the rest of the chromosome, as well as having a reduced diameter. Their texture was usually "smoother", less alveolar, with occasional indications of spiral structure. Short interstitial differential segments frequently appeared stretched to thin threads (Plate I fig. 1), as though the matrix were weakened or missing. When not stretched they might appear

simply as a crease across the chromosome.

Visible differentiation was produced by temperatures ranging from -2°C. to 13°C. It was first observed in metaphase chromosomes after half an hour at 3°C., which temperature was used for most experiments.

2. The Distribution of Differential Regions.

Each of the four species of Trillium studied was found to have a constant and characteristic distribution of differential regions in its chromosomes. The length of these regions was variable, but they could be classified in general terms as long or short. Differentially reactive segments having an interstitial position in the chromosome arms were usually short, while terminal ones tended to be longer.

In T. erectum it was found that at mitotic metaphase the chromosome complement had 42 potential differential regions (Text-fig. 1 and Table Ia), 32 interstitial and 10 terminal. No nucleus was found in which all 42 regions were differentiated. The numbers beside the differential regions in both Table I and Text-figs. 1 to 4 refer to their relative frequency of occurrence after 96 hours [illegible] at 3°C. In each chromosome region 1 was most frequently differentiated. The shaded segments although seen in many plants, were very rarely differentiated. In the E chromosome the two arms are so nearly the same length that it was impossible to distinguish

Table I

Number and position of differential segments

(a) T. erectum

Chromosome	Arm	Regions type	order[1]	Description
	Short	0		Arm sometimes pale.
A	Long	3I	2 3 1	In proximal 1/3 of arm. May merge to form one long region.
	Short	1T 2I	1 4	Usually 1/3 of arm. Adjacent to 1, may merge with it.
B			5	In proximal 1/3 of arm. Extremely rare.
	Long	2I	2 3	In proximal 1/4 of arm. 2 frequently long, 3 usually short.
	Short	1T	1	1/4 to 1/3 of arm.
C		1I	2	In proximal 1/3 of arm. Rare.
	Long	0		
	Short	1T	1	Variable in length, rarely more than 1/15 of arm.
D				
	Long	3I	2 4 3	In proximal 1/4 of arm. Very close together. Sub-terminal.
		1T	5	ca. 1/10 of arm.
	Short	1T	3	ca. 1/15 of arm. Extremely rare.
		2I	2 1	In proximal 1/4 of arm.
E				
	Long	3I	1a 2a 4	In proximal 1/3 of arm. 2a and 4 very close together 4 extremely rare.

Table I (cont'd)

(b) T. cernuum

Chromosome Arm	Regions: type	order[1]		Description
A	Short	0		
	Long	2I	1	In proximal 1/4 of arm.
			2	
B	Short	0		Arm short relative to the other species.
	Long	1I	1	In proximal 1/7 of arm.
C	Short	1I	1	At 1/3 of arm. Arm short relative to other species.
	Long	0		
D	Short	0		
	Long	0		
E	Short	1I	1	At 1/5 of arm.
	Long	1I	1a	At 1/6 of arm.

(c) T. grandiflorum

Chromosome Arm	Regions: type	order[1]		Description
A	Short	0		Arm sometimes pale.
	Long	2I	1	In proximal 1/3
			2	of arm.
B	Short	1T	1	Nearly 1/2 of arm.
	Long	0		
C	Short	1T	2	Very rare
		1I	1	Middle of arm.
	Long	0		
D	Short	0		
	Long	1I		In proximal 1/3 of arm.
E	Short	0		
	Long	0		

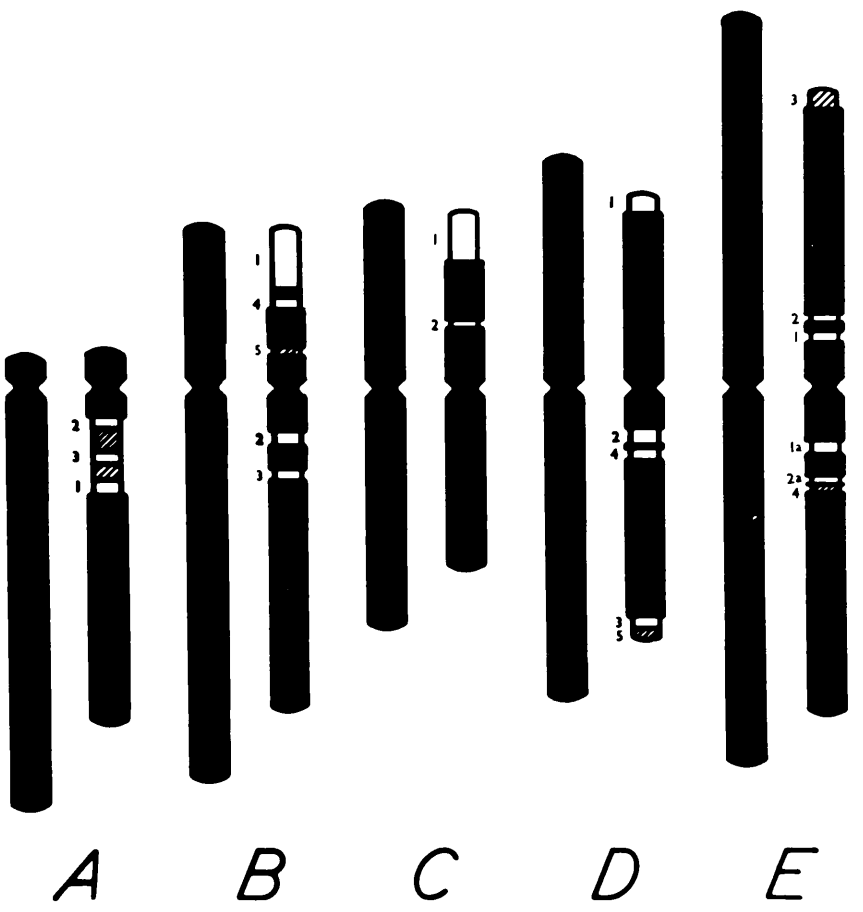
Table I (cont'd)

(d) T. undulatum

Chromosome Arm	Regions type	order[1]	Description
	Short	0	
A	Long	2I 1	In proximal 1/3 of arm.
		2	
	Short	1T 1	Usually 2/5 of trabant
B	Long	0	
	Short	3I	At 1/4, 1/2 and 3/4 of arm.
		?	Order not known.
C	Long	0	
	Short	0	
D	Long	0	
E	Short	0	
	Long	0	

Note: T = terminal differential region.
I = interstitial " "

[1] Numbers refer to relative frequency of occurrence within the chromosome after 96 hours at 3°C. Region 1 most frequent in each case.

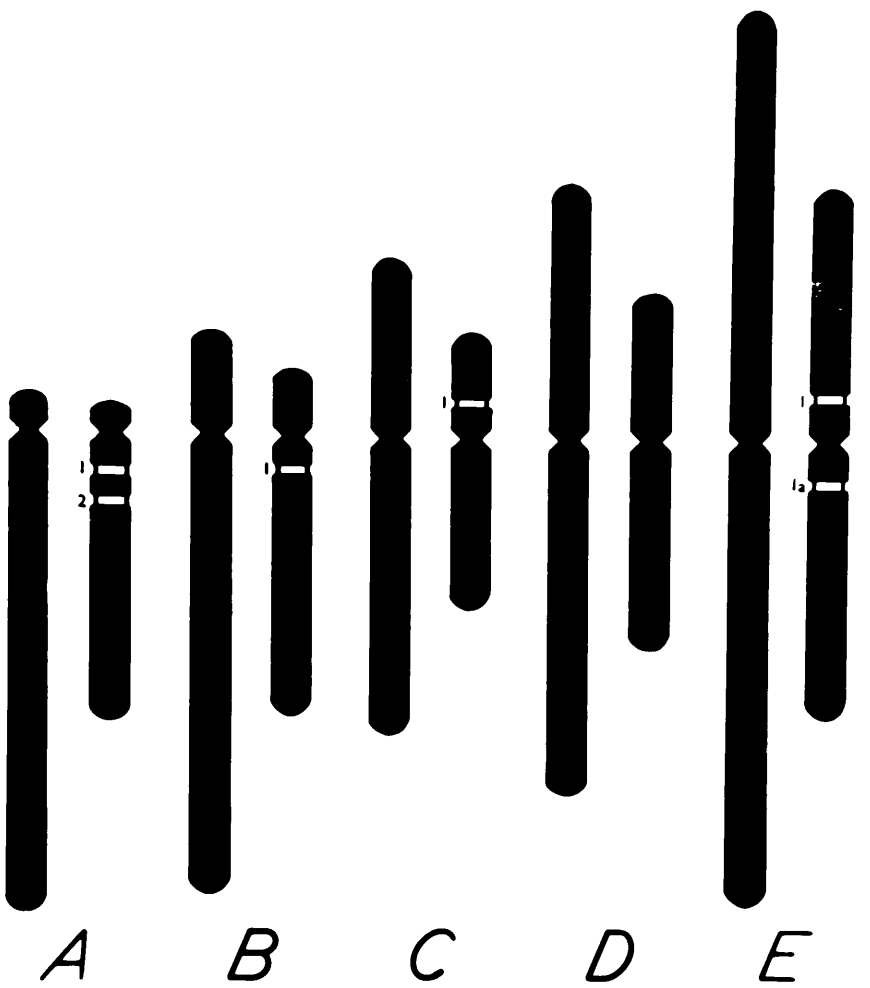


Text-fig. 1. A diagram showing the relative lengths of untreated and cold treated (96 hours at 3°C.) chromosomes, and the distribution of differential regions in Trillium erectum L. Numbers refer to the relative frequency with which the regions of each chromosome were differentiated (1 most frequent). Shaded regions were very rarely differentiated.

with certainty between regions 1 and 1a or 2 and 2a. The frequency of any one of these (e.g. of region 1) was therefore taken to be half the frequency of the pair of regions (1 and 1a). The placing of region 3 of the E chromosome in the shorter arm was purely arbitrary, since it has not been found frequently enough to make the measurements necessary to locate it with certainty.

Constant differences in differential regions were seen between plants of one species. These never took the form of differential segments in unusual positions, but were always quantitative. Plants were found in which one or more differential regions were missing, and others in which one of the terminal differential segments was always short. An analysis of certain of these cases will be presented later.

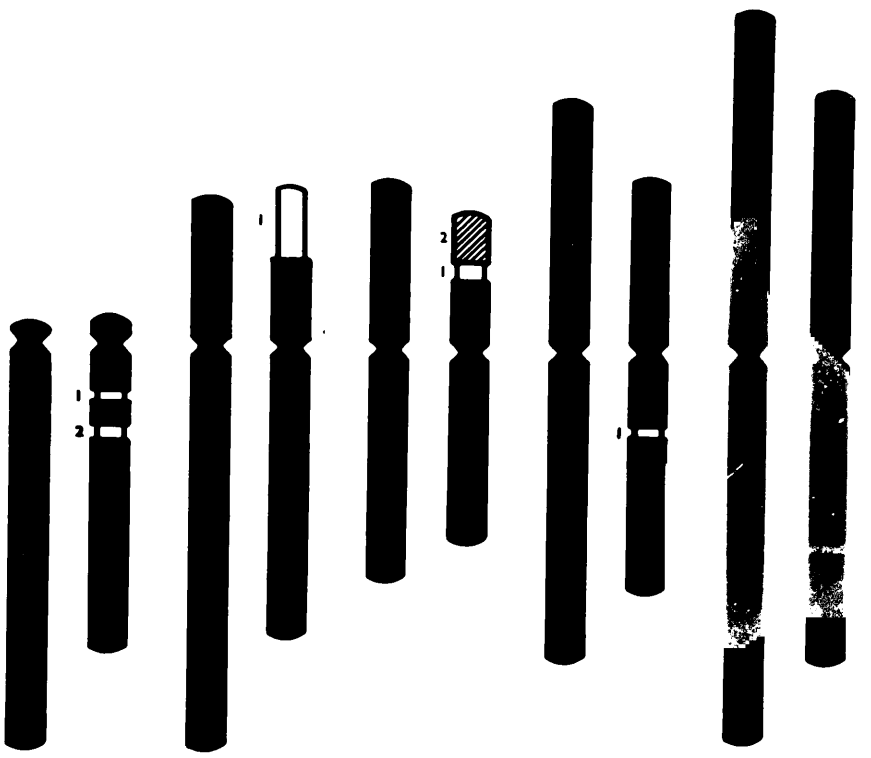
Not all of the differential regions remained discrete and separated by undifferentiated segments at all times. There was a "merging" of certain of them as the result of the "secondary differentiation" of the intervening parts of the chromosome. This was found most frequently in the A chromosome, where the three primary differential regions were sometimes united into one long region, and in the B, where regions 1 and 4 frequently merged (Plate I fig. 1). In the most extreme cases the secondary differentiation extended across the centromere of the A chromosome to include the whole of the short arm, from region 2 to the centromere in the B and D chromosomes, and across the centromere from region 1 to 1a in the E chromosome (Plate II fig.9). Except in the case of regions 1 and 4 of the B chromosome, the primary differential regions could always be distinguished because they exhibited a more extreme degree of differentiation than the secondary regions. The latter were not as narrow, and in general took a slightly darker stain than the former.



Text-fig. 2. A diagram showing the relative lengths of untreated and cold treated (96 hours at 3°C.) chromosomes, and the distribution of differential regions in Trillium cernuum L .

In T. cernuum the mitotic chromosomes were found to contain at metaphase 12 potential differential regions (Text-fig. 2 and Table Ib). Unlike those of T. erectum, none of these was terminal. All the regions were found to be very short, and within the 1/3 of the arm proximal to the centromere. After 96 hours at 3°C. they did not show much sharp differentiation from the rest of the chromosome as was usually found in T. erectum (Plate III fig. 11). Those arms which in T. erectum showed long terminal differential regions,

namely the shorter arms of the B and C chromosomes, were found to be relatively short in T. cernuum compared with other species.



Text-fig. 3. A diagram showing the relative lengths of untreated and cold treated (96 hours at 3°C.) chromosomes, and the distribution of differential regions in Trillium grandiflorum Salisb.

The root-tip metaphase chromosomes of T. grandiflorum were also found to contain 12 regions capable of differentiation from the rest of the chromosome (Text-fig. 3 and Table Ic). Eight of these regions were short interstitial ones, and four long terminals, one pair of the latter

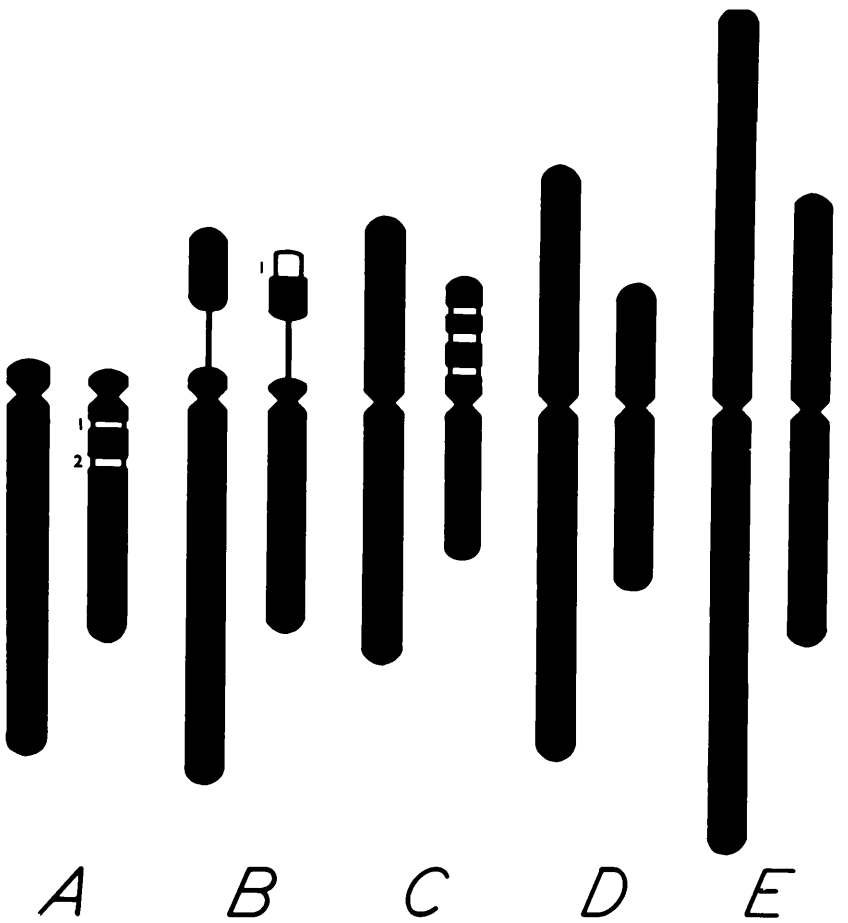
(in the C short arm) being very rarely differentiated. When differentiated, however, they were continuous with region 1 in the same arm. T. grandiflorum showed the same type of clear-cut differentiation as T. erectum (Plate III fig. 12).

The normal untreated chromosome set of T. undulatum has one morphological character which distinguishes it from those of the other species studied, namely a long secondary constriction in the B chromosome short arm. In this species also were found 12 potential differential regions per mitotic metaphase (Text-fig. 4 and Table Id), 10 interstitial and one pair of terminal regions in the trabants of the B chromosome. The three interstitial differential regions in the C short arm could not be classified for frequency since all three were differentiated in all C chromosomes of the material studied.

There was found to be some variation between plants in the relative frequency with which regions became differentiated. Consequently the figures for relative frequency which are given in Table I and Text-fig. 1 to 4 must be considered to represent only that order of differentiation most frequently observed. Details of the relative frequencies in T. erectum will be presented in the following section.

3. Changes in the Amount of Differentiation.

Visible differentiation was not found to be an all



Text-fig. 4. A diagram showing the relative lengths of untreated and cold treated (96 hours at 3°C.) chromosomes, and the distribution of differential regions in Trillium undulatum Willd.

or none" process for the nucleus as a whole. The number of differential regions observed in any nucleus after cold treatment varied from 0 up to the possible in some cases. In Trillium cernuum and T. undulatum certain nuclei showed all 12 possible regions, while the highest numbers observed in T. grandiflorum and T. erectum were 10 and 23 respectively. In T. grandiflorum, however, region 2 of the C chromosome was differentiated so rarely that for all practical purposes 10 regions per nucleus might be considered to be the

maximum. Although the number of differential regions showed some variation between plants and between nuclei within plants after the same treatment, most of the observed variations appeared to be the result of differences in the duration of cold treatment.

Two series of experiments were performed to determine the effect on the differentiation frequency first, of uniform cold treatment, and second, of cold followed by higher temperatures. In most of these experiments T. erectum was used because the high number of potential differential regions allows for greater variation. The results were checked by one or two experiments with T. grandiflorum, to get an indication of whether they were true only of the one species, or were of more general application. In these experiments the number of differential regions has been expressed as a percentage of an arbitrarily chosen "standard" number of regions -- 34 per nucleus in T. erectum and 10 in T. grandiflorum. These numbers represent all those regions which were differentiated with appreciable frequency after 96 hours at 3°C.

Uniform cold treatment. Plants of T. erectum were kept at a uniform temperature of 3°C., and root tips taken after various times from 1/4 hour to 2040 hours (ca. 12 weeks). The same plants could not be used throughout the experiment, since none was found with a sufficient number of

Table II

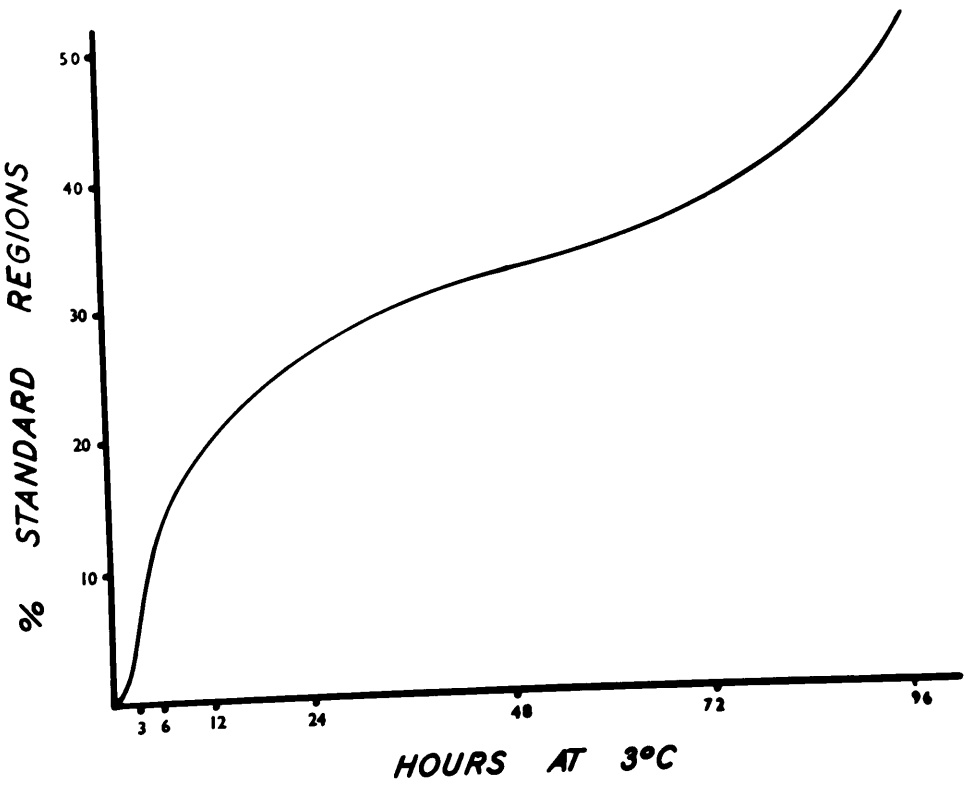
Percentage of "standard" regions differentiated in T. erectum, during cold treatment

Plant	Hours at 3°C. 1/4		1/2		1		3		6		12		24		48		72		96		168		240		336		672		1344		2040	
	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei
M							4	29	10	20	17	10	23	18	29	12	36	25	39	24												
O									11	10	19	9	26	20																
P	0	11	1	10	2	9	4	20											46	46										
Q											18	8	23	10			43	16	45	11										
R																			55	10					54	16	58	10		
D_2									22	10	19	16	35	16	37	10	38	12	45	14										
E_2									14	7	17	7	30	22			35	7												
F_2											15	6							40	22										
V_2																			53	20	44	15	42	20						
Y_2																			56	20	42	20	48	13	42	10				
B_4																			62	20					27	15	37	13	32	10		
C_4																			60	20					33	6	35	14	31	10		
D_4																			51	15					29	5	35	8	33	11	28	10
G_4																			52	20	47	12	49	20	45	13						
Mean	0		1		2		4		14		18		27		33		38		50		44		46		38		37		32		28	

Note: Percentages expressed as the nearest whole number.

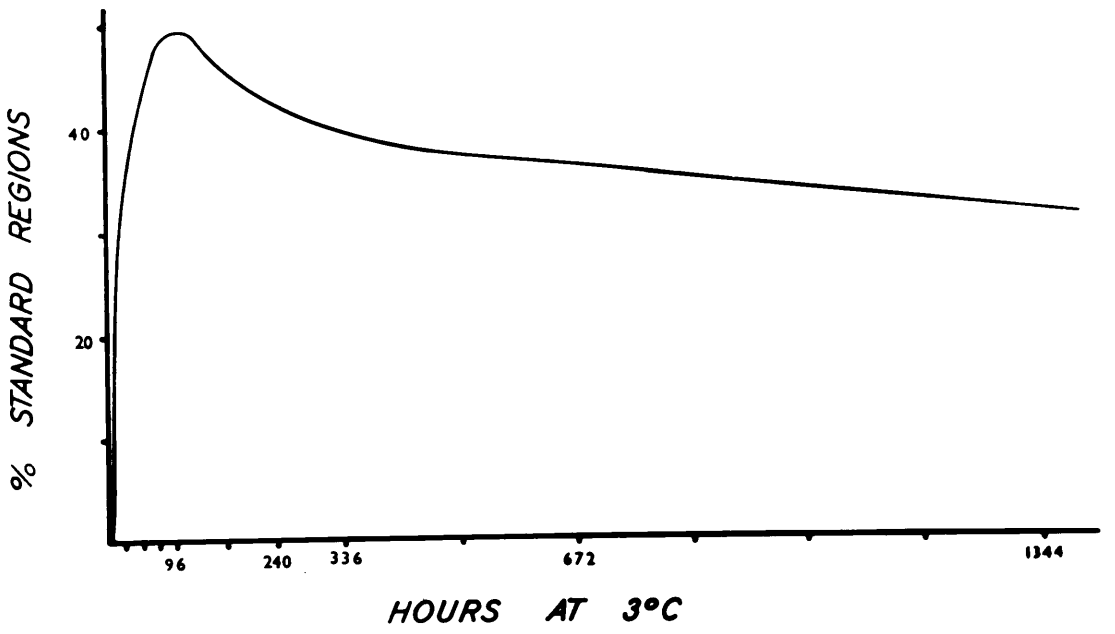
rapidly growing roots. Consequently, different plants were used for various parts of the range, and the ranges for the individual plants overlapped (see Table II).

At 3°C. the first differentiation was observed after half an hour, at which time 1% of the standard regions were differentiated (Table II). Thereafter the number of differential regions observed per cell increased rapidly for the first 96 hours at this temperature, at which time the maximum amount of differentiation for



Text-fig. 5. The degree of differentiation of the chromosomes of T. erectum for the first 96 hours at 3°C., based on the number of regions observed.

the conditions of the experiment appeared to have been reached (Text-fig. 5). After 96 hours at 3°C. the observed frequency of differential regions decreased with continued cold treatment. This decrease, at first relatively rapid, became slow and nearly uniform from 336 to 2040 hours, (see Table II and Text-fig. 6). by which time mitoses were so scarce that the experiment was discontinued.

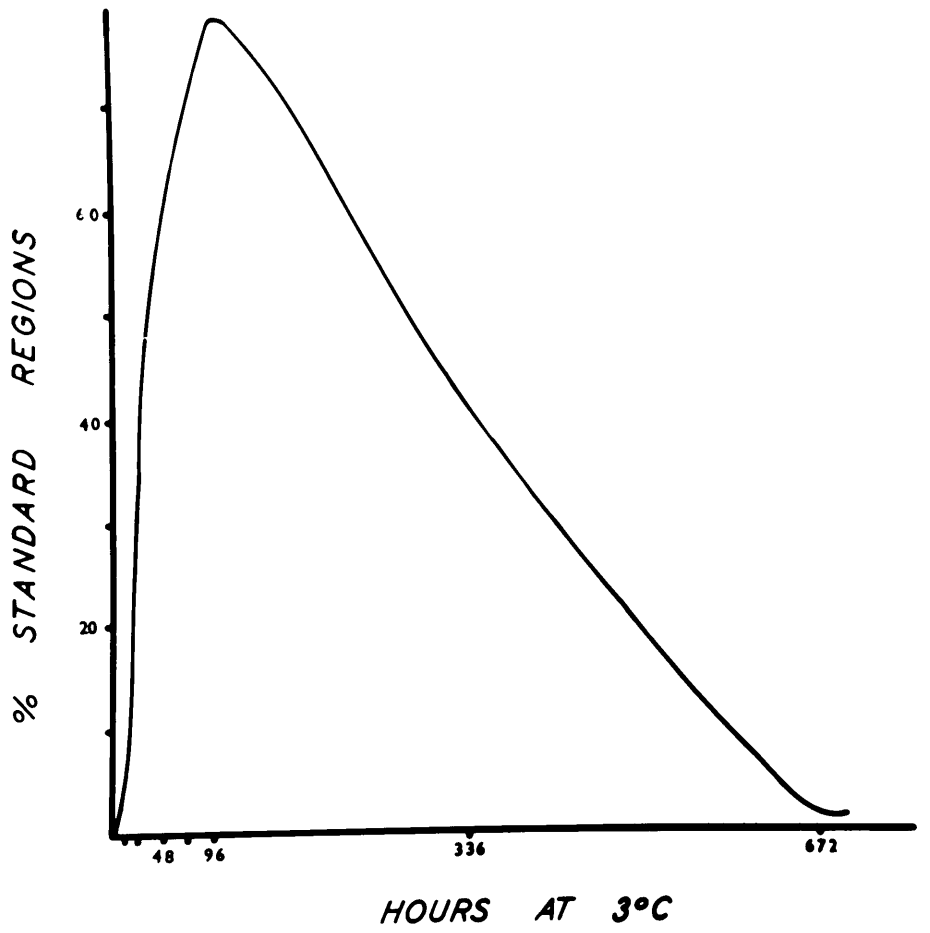


Text-fig. 6. The degree of differentiation of the chromosomes of T. erectum during 1344 hours at 3°C., based on the number of regions observed.

It is obvious from this experiment that at 3°C. the chromosomes do not steadily increase in differentiation with time until the maximum possible number of regions is differentiated. On the contrary, there is an increase to a

maximum well below the possible, in fact only about 40% of the potential or 50% of the standard number of regions, and then the chromosomes tend to return towards their usual undifferentiated condition (Plate II figs. 3 to 8).

A second experiment, similar to but less extensive than that just reported, was performed on T. grandiflorum. Root tips were taken after 3 to 1000 hours at 3°C., and again it was necessary to use plants in an overlapping series. Table III and Text-fig. 7 show the degree of



Text-fig. 7. The degree of differentiation of the chromosomes of T. grandiflorum, at 3°C., based on the number of regions observed.

Table III

rcentage of "standard" regions differentiated, in T. grandiflorum, during cold treatment

ant	Hours at 3°C. 3		6		12		24		48		72		96		336		672		1000
	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	% No. Nuc.
	2	11			4	7	27	16											
					3	8	24	20	46	8	65	12	72	13					
	2	6																	
Q					2	13	15	18											
W			8	6									66	16					
Z									69	11	59	18							
A_2							45	11											
B_2							60	15	69	8	86	12	81	20					
D_2	4	20																	
E_2			3	20															
G_2											64	20					3	12	
H_2																	1	15	12 14
J_2			12	20															
X_2													94	5	54	11			
Y_2													80	7	30	11			
Mean	3		7		3		34		61		69		79		42		2		12

Note: Percentages expressed as the nearest whole number.

differentiation. Here, as in T. erectum, the number of differential regions observed per nucleus increased rapidly to a maximum for the experiment after 96 hours. This maximum, 79% of the standard or 66% of the possible differential regions, is relatively much higher than that in T. erectum although the actual number of regions was less. Subsequent to 96 hours, however, the two species differed radically. In T. grandiflorum the chromosomes appeared to have virtually regained their original undifferentiated condition after 672 hours, although retaining some differentiation after 1000 hours. In T. erectum, on the other hand, 37% of the standard number was observed after 672 hours, and 32% after 1344 hours. Unfortunately not sufficient data were obtained from T. grandiflorum to be certain of the results.

In both these experiments the degree of differentiation has been recorded on the basis of the number of differentiated regions observed per nucleus. Parallel with the changes in the number of regions a change in the sharpness of the differentiation was also seen (Plate II figs. 4-8). With the shortest and longest cold treatments the differential regions stained nearly as darkly as the rest of the chromosome, but were slightly smaller in diameter.

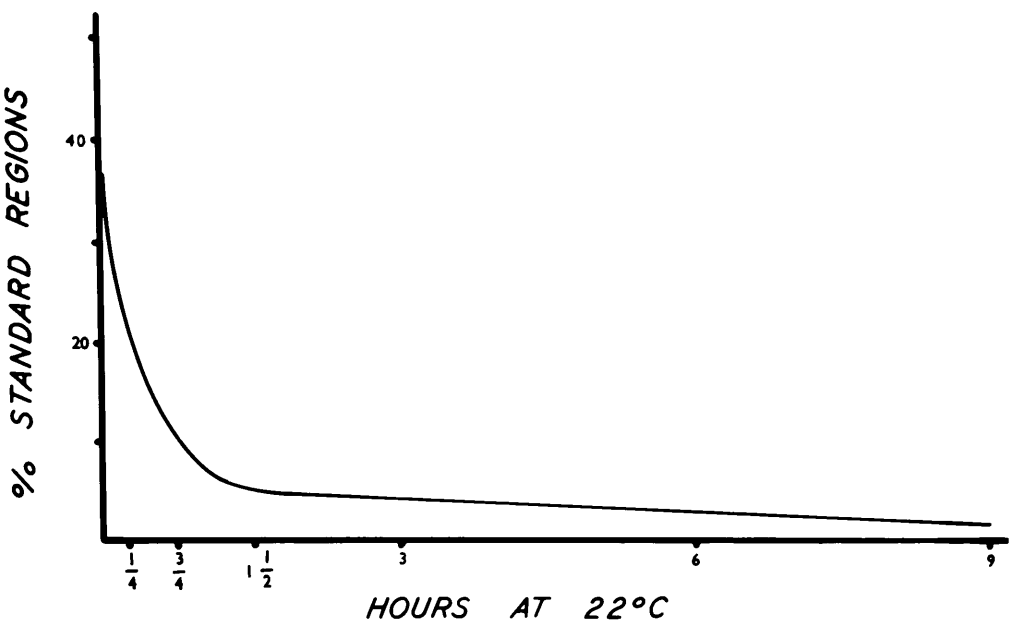
No experiments of this type were performed on either T. cernuum or T. undulatum. Observations after 96 hours at 3°C., however, showed 41% of the possible regions differentiated in the former and 98% in the latter.

"Recovery". When plants were returned to room temperature after cold treatment it was found that the mitotic chromosomes regained their original undifferentiated condition. This process of de-differentiation will be referred to as "recovery". Four recovery experiments were performed on T. erectum in which two factors were varied, namely the duration of the original cold treatment and the temperature at which recovery occurred. In the first three experiments recovery occurred at 22°C. after 24, 96 and 240 hours at 3°C. In the fourth recovery occurred at 35°C. after 96 hours at 3°C.

Table IV

Percentage of "standard" regions in T.erectum during "recovery" at 22°C. after 24 hours at 3°C.

	Hours at 22°C. after 24 hours at 3°C.											
	0		3/4		1 1/2		3		6		9	
Plant	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei
A_3	36	15			2	20			7	20	1	15
B_3	46	10	7	20	3	11			2	20		
C_3	32	20			7	20	8	10	3	20	5	20
D_3	34	11	9	15			4	20	3	20	1	15
E_3							4	13	4	10	0	10
Mean	37		8		4		5		4		2	



Text-fig. 8. The degree of differentiation of the chromosomes of T. erectum, based on the number of regions observed, during "recovery" at 22°C. after 24 hours at 3°C.

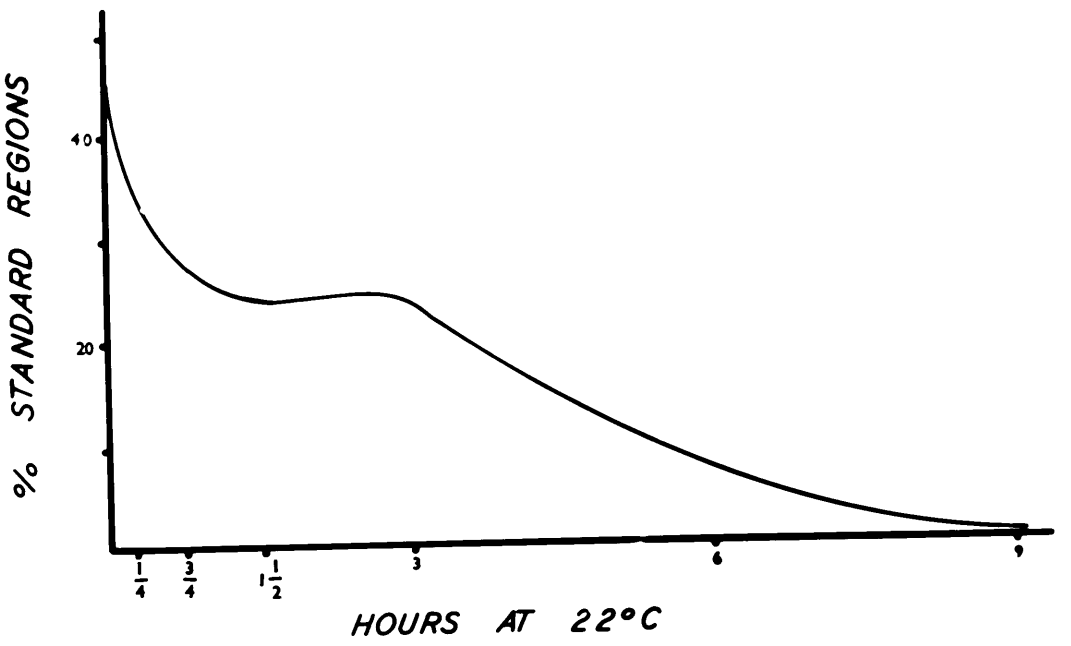
(1) After 24 hours at 3°C. 37% of the standard regions were differentiated (this is somewhat higher than previously reported, probably as the result of slight differences in plants and environment). The plants were then transferred to 22°C., where after 3/4 hour only 8% of the standard regions remained differentiated. After 1 1/2 hour 4% and after 9 hours 2% standard were observed (Table IV and Text-fig. 8). Recovery was very rapid at first, but was not complete in 9 hours.

Table V

Percentage of "standard" regions in T. erectum during "recovery" at 22°C. after 96 hours at 3°C.

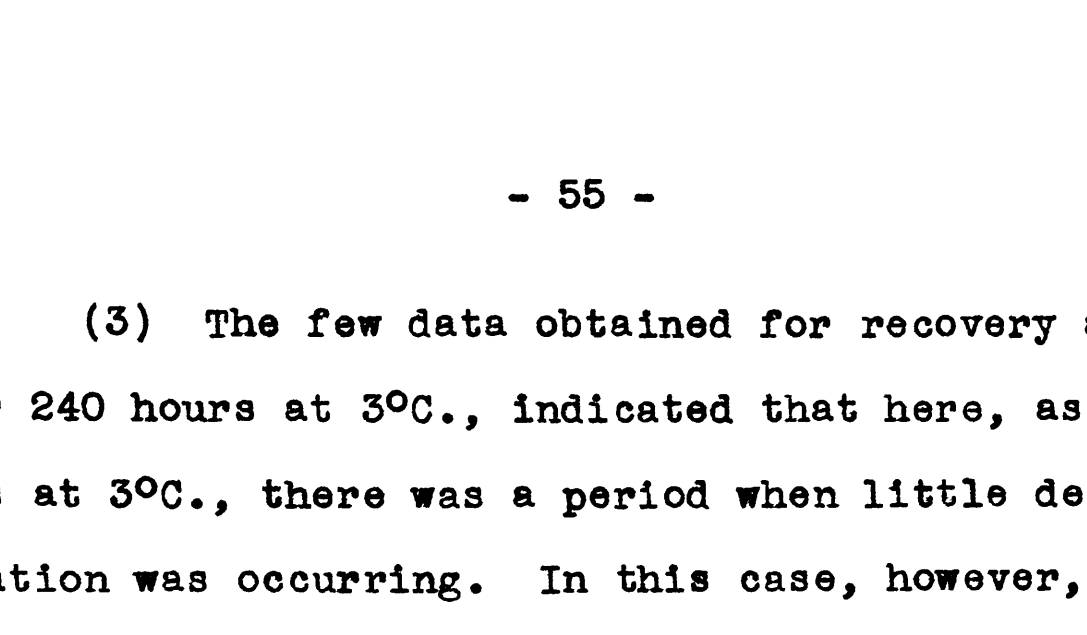
Plant	Hours at 22°C. after 96 hours at 3°C.																	
	0		1/4		3/4		1 1/2		3		6		9		12		24	
	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei
U_2	51	20	30	15	27	9											0	15
V_2	40	5															0	15
K_3	47	11	10	6	33	20									0	10	0	20
L_3	52	11	41	10	42	20	41	14	34	13					0	12	0	15
N_3	46	20	40	20							17	12						
Q_3	44	10			30	15	19	15	18	15					3	10		
R_3	46	15	35	20	25	20	12	20	12	11								
S_3	39	20									5	15						
T_3	42	20									4	20	2	10				
U_3	55	11							30	20	4	17	0	20	0	15		
Mean	46		31		31		24		24		8		1		1		0	

(2) The course of recovery after 96 hours at 3°C. was very different from this (Table V and Text-fig.9). Rapid recovery for the first 1 1/2 hours at 22°C. (from 40% to 24% standard) was followed by a further 1 1/2 hour period with apparently no more de-differentiation. The number of recognisable differential regions then decreased slowly until by 24 hours none remained.



Text-fig. 9. The degree of differentiation of the chromosomes of T. erectum, based on the number of regions observed, during "recovery" at 22°C. after 96 hours at 3°C.

(3) The few data obtained for recovery at 22°C. after 240 hours at 3°C., indicated that here, as after 96 hours at 3°C., there was a period when little de-differentiation was occurring. In this case, however, this period was from 3 /4 hour to 6 hours after the cessation of cold treatment, during which time recovery was very slight. Recovery was more rapid from 6 to 9 hours, but 1% of the standard regions was seen even after 24 hours at 22°C. (Table VI and Text-fig. 10).



Text-fig. 10. The degree of differentiation of the chromosomes of T. erectum, based on the number of regions observed, during "recovery" at 22°C. after 240 hours at 3°C.

Table VI

Percentage of "standard" regions in T. erectum during "recovery" at 22°C. after 240 hours at 3°C.

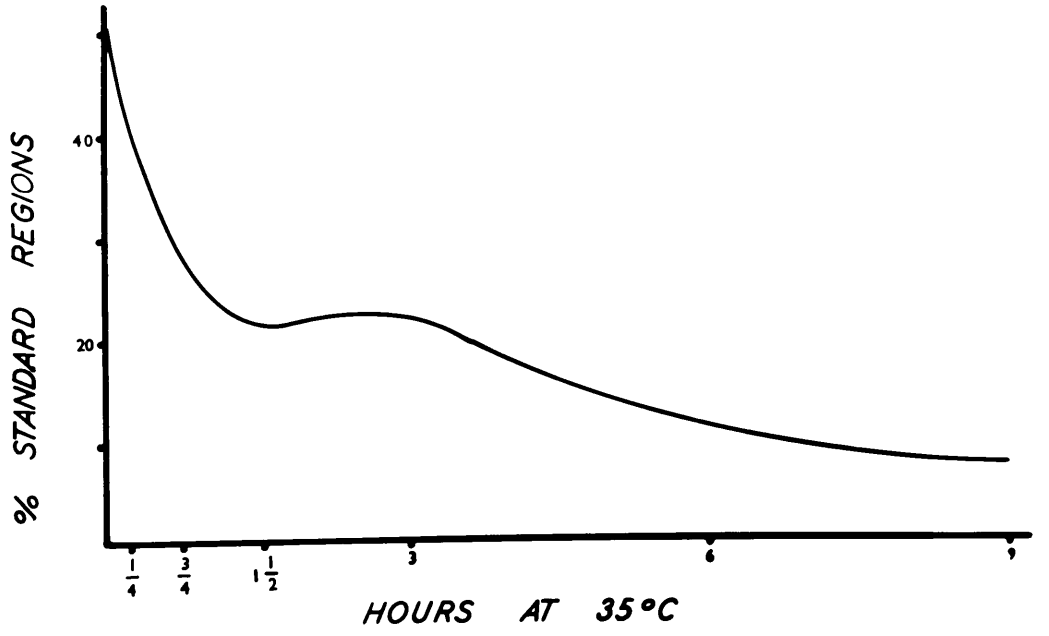
Plant	Hours at 22°C. after 240 hours at 3°C. 0		3/4		1 1/2		3		6		9		24	
	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei
C_5	41	2	35	3	35	5	32	2			6	3		
D_5	46	5	33	1	24	4	28	3	26	4				
E_5	36	1	33	1	40	1	29	3						
G_5	52	1												
O_5	52	1							17	1			1	5
Mean	45		34		32		29		24		6		1	

Table VII

Percentage of "standard" regions in T. erectum during "recovery" at 35°C. after 96 hours at 3°C.

Plant	Hours at 35°C. after 96 hours at 3°C. 0		3/4		1 1/2		3		9	
	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei	%	No. Nuclei
V_3	48	20	29	20	23	15	20	11	13	8
W_3	71	15	40	20						
Y_3	43	15							2	15
Z_3	43	16			19	13	23	15		
A_4	40	20	17	11						
Mean	51		29		21		22		8	

(4) In the fourth experiment temperature was varied. After 96 hours at 3°C. recovery occurred at 35°C. The course of recovery at this temperature was found to follow very closely that at 22°C. after the same cold treatment (compare Table VII and Text-fig. 11 with Table V and Text-fig. 9). Rapid initial recovery was followed by a period of no change, then a further falling off in the degree of differentiation. The percentage standard was somewhat higher after 9 hours at this temperature than at 22°C.



Text-fig. 11. The degree of differentiation of the chromosomes of T. erectum, based on the number of regions observed, during "recovery" at 35°C. after 96 hours at 3°C.

These four experiments suggest that chromosomes which have not yet reached their maximum degree of differentiation recover very rapidly at high temperatures, while those which have reached or passed the maximum recover more slowly, passing through a period of very little change. In all cases differentiation appeared to be virtually complete after 12 to 24 hours at the higher temperature.

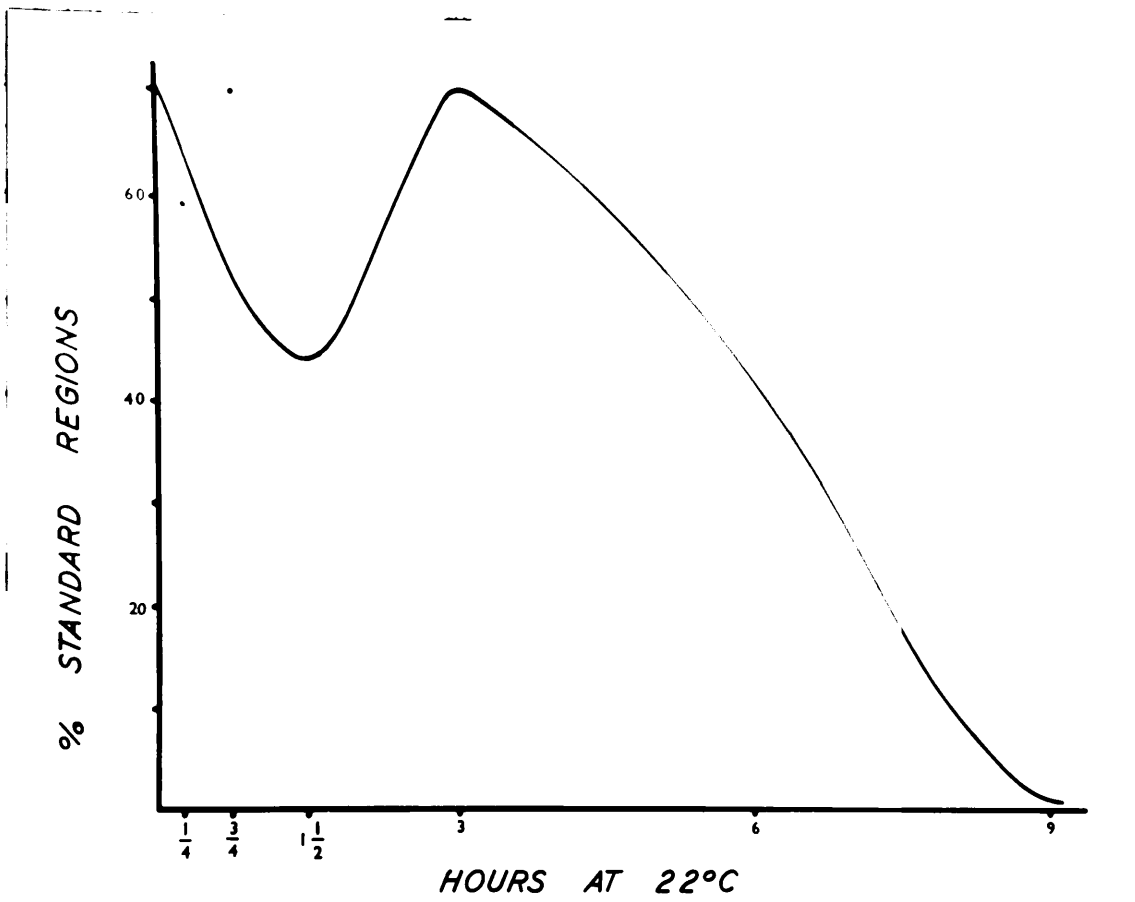
Five plants of T. grandiflorum were used for a fifth recovery experiment to check the results obtained with T. erectum. Recovery occurred at 22°C. after 96 hours at 3°C. Very few mitoses were found, and the results obtained were mainly from a single plant, from which, unfortunately, no zero point could be obtained. The results, therefore, can only be interpreted in very general terms. The percentage of standard regions decreased, with some fluctuations, for the first 1 1/2 hour at 22°C., after which there was a sharp increase to 3 hours, followed by a steady decrease, until by 9 hours recovery was almost complete (Table VIII and Text-fig. 12). The time required for complete recovery was similar to, or slightly less than that for T. erectum. The fluctuations suggest that a more extensive investigation might yield interesting results.

Variability. The same number of differential regions was

Table VIII

Percentage of "standard" regions in T. grandiflorum during "recovery" at 22°C. after 96 hours at 3°C.

Plant	Hours at 22°C. after 96 hours at 3°C. 0		1/4		3/4		1 1/2		3		6		9		12	
	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei	No.	% Nuclei
A			63	20	65	15	44	15	71	20	35	4	1	10	0	11
O_2	86	15			79	7										
P_2	75	4													0	6
Q_2	61	15	47	7												
T_2	66	20									45	11				
Mean	71		59		70		44		71		42		1		0	



Text-fig. 12. The degree of differentiation of the chromosomes of T. grandiflorum, based on the number of regions observed, during "recovery" at 22°C. after 96 hours at 3°C.

not observed in all plants of one species after the same duration of cold treatment. Thus, in Table II it may be seen that after 96 hours at 3°C. different plants showed from 39% to 62% of the standard number of regions differentiated. The differences could not be accounted for by the absence, in some plants, of certain specific differential regions, but appeared to be the result of general differences in the degree of differentiation. Two facts indicate that these differences are probably not caused by genetic

variation between plants, since those which show a high percentage of differential regions at one time may show a low percentage at another (cf. B_4 and G_4 at 96 and 336 hours, Table II). Plant D_4 was used in two experiments several months apart. In these two experiments the percentage of standard regions was almost the same after 96 hours (51% and 52%), but widely different after 336 hours (29% and 45%). It seems probable that the physiological condition of the plant, or even of the individual root tip, at the time of the experiment determines the extent of the response to reduced temperature. Slight environmental differences during the experiment might also be determining factors.

It has already been stated that the individual regions were not all differentiated with the same frequency after 96 hours at 3°C. (see Table I and Text-figs. 1 to 4). Table IX gives the mean frequencies with which the regions of all four species were observed after this treatment. In T. erectum the mean frequencies varied from less than 1% to 99%, and for individual plants 100% of the possible. The distribution was not continuous, however, no regions having been found with mean frequencies between 21% and 65%. In T. grandiflorum the range was from 4% to 100%, in T. cernuum 23% to 64%, and in T. undulatum only 90% to 100%. The true variation may be slightly less than this apparent one, since

Table IX

The frequency of appearance of the differential regions in four species of Trillium after 96 hours at 3°C.

Individual regions differentiated (%)	T.erectum	T.cernuum	T. grandiflorum	T.undulatum
100			B-1	A-1,B-1,C(3)
99	B-1			
96	A-1			
91	E-1 & 1a			
90				A-2
87	C-1			
80	B-2			
78	D-1			
72			C-1	
70	D-2			
66			D-1	
65	A-2			
64		A-1		
56			A-1	
55		B-1		
41		E-1 &1a		
36			A-2	
23		C-1,A-2		
21	E-2 &2a			
19	D-3			
14	B-3			
12	A-3			
9	D-4			
6	C-2			
5	B-4			
4				C-2
2	D-5			
< 1	E-3 & 4,B-5			

obviously not all regions can be distinguished with equal ease. However, the lack of direct correlation between the length of the differential regions and the frequency with which they were observed, and the fact that their order of frequency is not the same in all plants indicate that not all the variation can be ascribed to difficulties of observation.

4. Length Relationships in the Chromosomes.

Differentiation of the somatic chromosomes was never observed at early prophase stages, when contraction is just beginning, but only from late prophase to telophase, during which time the contraction of the chromosomes is greatest. It seemed probable, therefore, that the degree to which the chromosomes were contracted might play some part in the expression of differential reactivity.

The length of the chromosome complement. The total length of the somatic metaphase chromosomes did not differ very greatly between the four Trillium species when the plants were grown at room temperature, and in all cases lower temperatures caused an increased contraction (Table X). The relative lengths of the chromosomes at 22°C. and after 96 hours at 3°C. are represented graphically in Text-figs. 1 to 4. The degree of contraction of the chromosomes of T. erectum during cold treatment was found to follow a trend

Table X

Length, in micra, of the chromosome complement during cold treatment

Hours at 3°C		0	12	24	72	96	168	240	336	672
T.erectum	Length	285	283	255		234	212	178	193	254
	σ_m	±6.9	±13.0	±6.9		±4.5	±7.5	±4.8	±6.5	±5.9
	No.of Nuclei	30	10	20		25	15	20	14	10
T.cernuum	Length	313				187				
	σ_m	±18.2				±2.9				
	No.of Nuclei	7				11				
T.grandiflorum	Length	298				211	211		218	
	σ_m	±12.2				±7.4	±6.5		±5.3	
	No. of Nuclei	26				15	25		25	
T.undulatum	Length	293							172	
	σ_m	±19.8							±6.1	
	No.of Nuclei	5							10	

similar to that for the frequency of differential regions. However, the relationship between differentiation and contraction was not found to be a simple case of the number of differential regions observed being inversely proportional to the length of the chromosome complement. Maximum differentiation was observed after 96 hours at 3°C., but maximum contraction at that temperature not until 240 hours. After the maximum was reached both contraction and differentiation decreased with continued cold treatment.

Measurements of chromosome length in T. erectum during "recovery" also showed that the relationship between the amount of differentiation and the length of the chromosomes was not one of simple inverse proportion, as had at first seemed probable (see Table XI). After 96 hours at 3°C. the length of the chromosomes decreased for the first 1 1/2 hour at 22°C. For this range, therefore, there was a direct correlation between length and differentiation. After 1 1/2 hour the length gradually increased and had reached its normal value for 22°C. after 24 hours (the high value at 9 hours is probably very inaccurate). With the exception of 1 1/2 to 3 hours, when no change in the amount of differentiation was observed, increased chromosome length was accompanied by decreased differentiation. The few measurements obtained from material which recovered at 35°C. after 96 hours at 3°C. suggested that the chromosome

Table XI

Length of the chromosome complement in T.erectum during "recovery"

	Hours after cessation of cold treatment								
22°C. after 24 hrs.at 3°C.	0	1/4	3/4	1 1/2	3	6	9	12	24
Length	255		271	204	242				
σ_m	±6.9		±8.5	±14.2	±13.6				
No.of Nuclei	20		10	10	5				
22°C.after 96 hrs.at 3°C.									
Length	234	209	195	183	209	229	285	252	296
σ_m	±4.5	±7.2	±4.6	±4.5	±5.9	±7.4	±25.7	±9.0	±6.5
No.of Nuclei	25	20	20	20	20	20	5	15	10
35°C.after 96 hrs.at 3°C.									
Length	234		194	194					
σ_m	±4.5		±10.5	±7.6					
No.of Nuclei	25		15	5					

lengths would probably be similar at that temperature. After 24 hours at 3°C. minimum length was again observed after 1 1/2 hour at 22°C., at which time differentiation was very slight (4% standard). From these measurements it is apparent that differentiation, although related to chromosome contraction, shows no simple correlation with the length of the metaphase complement.

The relative length of chromosome arms. Since total chromosome lengths gave only very limited information on the relationship between differentiation and contraction, the relative lengths of the chromosome arms were determined both in differentiated and undifferentiated material, in the hope that these might furnish a clue to the true relationship. With the exception of the A and E chromosomes, for which the total length was used, the mean lengths of the chromosome arms for all four species, expressed as percentages of the total length of the complement, are given in Table XII for both untreated material and that kept 96 hours at 3°C. Examination of the table shows immediately that after cold treatment the greatest relative increase in length (i.e. the least contraction) occurred in those arms with the greatest amount of differentiation, namely the B and C short arms in T. erectum, the B short arm in T. grandiflorum, and the A chromosome and B and C short arms in T. undulatum. The few measurements of this last species and their high variability

Table XII

Lengths of chromosomes and chromosome arms, expressed as percentages of the total length of the complement, in undifferentiated and highly differentiated material from the four species

Species and Treatment	A Chromosome	B Long	B Short	C Long	C Short	D Long	D Short	E Chromosome	Total Length
T. erectum									
Untreated	15.7	14.5	5.9	8.6	6.7	11.3	8.6	28.2	285 μ
96 hrs. 3°C.	16.0	14.0	7.3	8.0	7.9	11.1	8.5	27.1	234 μ
difference	+0.3	-0.5	+1.4	-0.6	+1.2	-0.2	-0.1	-1.1	
T. cernuum									
Untreated	16.9	14.9	3.5	9.7	5.9	11.6	8.3	29.2	313 μ
96 hrs. 3°C.	17.3	15.1	3.6	9.4	5.8	11.5	8.1	29.2	187 μ
difference	+0.4	+0.2	+0.1	-0.3	-0.1	-0.1	-0.2	0.0	
T. grandiflorum									
Untreated	15.4	14.5	5.9	9.4	6.9	11.4	8.7	27.7	298 μ
96 hrs. 3°C	16.0	13.8	7.7	9.2	6.6	11.4	8.3	27.0	218 μ
difference	+0.6	-0.7	+1.8	-0.2	-0.3	0.0	-0.4	-0.7	
T. undulatum									
Untreated	14.1	13.7	4.0[1]	9.4	6.7	12.8	8.7	30.0	293 μ
96 hrs. 3°C.	16.4	14.1	5.3[1]	9.5	7.9	11.1	7.8	27.8	172 μ
difference	+2.3	+0.4	+1.3	+0.1	+1.2	-1.7	-0.9	-2.2	

1 Variable secondary constriction not included in measurements.

in the untreated material (see Table X) make it unreliable. No large changes in relative length were found in T. cernuum, in which species also no large differential regions were observed. This analysis indicated that relative rather than absolute lengths were of importance in the investigation of differential reactivity.

Because the long arm of the C chromosome was never differentiated it has been used, in place of the total length of the complement, as the standard of comparison in Table XIII. Here, the length of the chromosome arms of T. erectum and T. grandiflorum have been expressed as percentages of the corresponding C long arms. The material has been grouped solely by the percentage of differential regions observed, regardless of the treatment it had received. Thus, for T. erectum, the low differentiation class includes material kept 12 hours at 3°C. and 6, 9, 12 and 24 hours at 22°C. after 96 hours at 3°C.. The high differentiation class includes material kept 96, 168, 240, 336 and672 hours at 3°C. The high differentiation class for T. grandiflorum includes material kept 72 and 96 hours at 3°C.. No measurements of material with a low degree of differentiation were available for this species.

The data presented in Table XII suggested several predictions of the results to be expected from the analysis recorded in Table XIII. In the comparison of highly

Table XIII

Chromosome and chromosome arm lengths expressed as percentages of the long arm of the C chromosome

Percentage of standard regions	A Chromosome	B Long arm	B Short arm	C Short arm	D Long arm	D Short arm	E Chromosome
T. erectum							
Untreated 0%	192 ± 6.4	167 ±5.1	69 ± 2.6	77 ± 2.8	131 ± 4.6	100 ± 3.3	332 ± 11.0
Low 1% - 19%	185 ± 4.7	162	70	85	131	100	314 ± 8.2
High 35% - 50%	207 ± 4.1	178 ±3.8	83 ± 1.9	89 ± 2.0	140 ± 2.8	109 ± 2.7	339 ± 7.5
T.grandiflorum							
Untreated 0%	164 ± 7.4	154 ±3.4	63 ± 2.8	73 ± 3.7	121 ± 5.4	93 ± 4.6	294 ± 12.1
High 69% - 79%	172	151	87	74	127	90	295

differentiated with undifferentiated chromosomes no arm should show a decrease in length relative to the C long arm. The greatest relative increase in T. erectum should be in the B and C short arms, and the least in the E chromosome. In T. grandiflorum the greatest relative increase should be in the B short arm, with none in the B long and D short arms or the E chromosome. Most of these predictions were fulfilled by the data in Table XIII. The higher the proportion of the arm which was differentiated the greater was its length relative to the C long arm.

The length of differential and non-differential segments.

In the last two tables comparisons were made between the lengths of chromosome arms which contained both differential and non-differential parts, but no comparison was made between completely differentiated and completely undifferentiated segments. The short arms of the B and C chromosomes of T. erectum and the B chromosome of T. grandiflorum all contain terminal differential regions (number 1 in each case; see Text-figs. 1 and 3) long enough for such a comparison. The remaining parts of these arms include other differential regions, but since all of these are very short and seldom differentiated they have been disregarded in the present analysis, and these parts of the arms have been considered to be non-differential. The lengths of these differentiated and non-differentiated

parts of the short arms, and of the C long arms have been recorded in Table XIV for both species during cold treatment, and for T. erectum during recovery at 22°C. after 96 hours at 3°C.

From 12 to 96 hours at 3°C., both B and C differential regions of T. erectum increased in absolute length. Longer periods at this temperature caused them once again to become more contracted. The non-differential parts of the arms behaved in a different manner, similar to the C long arm. All of these contracted steadily for 240 hours at 3°C., after which time they once again elongated. The similarity in the behaviour of these three segments suggests that probably all non-differential parts react to cold in essentially the same manner. This is further supported by the fact that the length of the chromosome complement, which is composed largely of non-differential chromatin, changed in like manner at 3°C. (see Table X). The few data in T. grandiflorum show that the B chromosome differential obtained maximum length after 72 hours at 3°C. The two arms of the C chromosome contracted for 24 hours,after which they and the B non-differential segment showed a very slight increase to 96 hours.

During recovery at 22°C. after 96 hours at 3°C. all segments first contracted, then elongated. However, the differential regions attained minimum length after 3/4 hour at 22°C., after which there was only a very slight

Table XIV

Lengths of differential and non-differential segments in the B and C chromosomes, in micra, during cold treatment and "recovery".

T. erectum

Hours at 3°C.	B chromosome Short arm Diff.region $\bar{X} \pm \sigma_{\bar{x}}$	B chromosome Short arm Non-diff.reg. $\bar{X} \pm \sigma_{\bar{x}}$	B chromosome Total $\bar{X}$	N	C chromosome Short arm Diff.reg. $\bar{X} \pm \sigma_{\bar{x}}$	C chromosome Short arm Non-Diff.reg. $\bar{X} \pm \sigma_{\bar{x}}$	C chromosome Total $\bar{X}$	N	Long arm Total $\bar{X} \pm \sigma_{\bar{x}}$	N
0			8.42±0.22	60			9.41±0.21	60	12.22±0.28	60
12	2.92±0.35	5.94±0.56	8.86	13	2.02±0.18	7.58±0.29	9.60	9	12.10±0.68	20
24	3.04±0.17	5.10±0.19	8.14	38	2.14±0.15	7.34±0.30	9.48	30	11.00±0.28	40
96	3.68±0.16	4.96±0.16	8.64	50	2.52±0.15	6.66±0.19	9.18	46	9.40±0.25	50
168	3.28±0.12	4.08±0.18	7.36	25	2.12±0.18	5.70±0.22	7.82	21	8.32±0.26	30
240	2.86±0.15	3.58±0.14	6.44	32	1.88±0.12	4.80±0.14	6.68	30	7.14±0.12	40
336	2.70±0.13	3.93±0.13	6.63	26	2.10±0.27	5.13±0.25	7.23	12	7.71±0.32	28
672	3.10±0.15	5.48±0.31	8.58	18	1.88±0.20	7.86±0.41	9.74	8	10.12±0.42	20
Hours at 22°C. after 96 hours at 3°C.										
0	3.68±0.16	4.96±0.16	8.64	50	2.52±0.15	6.66±0.19	9.18	46	9.40±0.25	50
1/4	2.56±0.13	4.23±0.16	6.79	40	2.66±0.19	5.71±0.16	8.37	36	8.86±0.32	40
3/4	2.10±0.22	4.18±0.15	6.28	39	2.00±0.24	5.70±0.15	7.70	36	8.21±0.20	40
1 1/2	2.17±0.14	3.82±0.11	5.99	29	2.43±0.18	5.45±0.21	7.88	29	7.56±0.20	40
3	2.39±0.18	4.39±0.16	6.78	38	2.37±0.15	5.32±0.13	7.69	38	8.74±0.27	40
6	2.57±0.20	5.06±0.15	7.63	31	2.36±0.31	6.76±0.14	9.12	25	9.76±0.30	40

Table XIV (cont'd)

1. grandiflorum

Hours at 3°C.	B chromosome Short arm Diff.region $\bar{X} \pm \sigma_{\bar{x}}$	B chromosome Short arm Non-diff.region $\bar{X} \pm \sigma_{\bar{x}}$	B chromosome Total $\bar{X}$	N	C chromosome Short arm Total $\bar{X} \pm \sigma_{\bar{x}}$	N	C chromosome Long arm Total $\bar{X} \pm \sigma_{\bar{x}}$	N
0			8.8 ± 0.3	52	10.2 ± 0.4	52	14.0 ± 0.5	52
24	2.8 ± 0.2	4.2 ± 0.1	7.0	26	6.8 ± 0.3	30	9.6 ± 0.3	30
72	4.4 ± 0.2	4.4 ± 0.2	8.8	50	7.2 ± 0.1	50	9.6 ± 0.2	50
96	3.8 ± 0.1	4.6 ± 0.1	8.4	50	7.2 ± 0.2	50	10.0 ± 0.2	50

increase by 6 hours. The non-differential parts, on the other hand, did not attain minimum length until 1 1/2 to 3 hours, and showed a considerable increase by 6 hours. Here also the total length of the complement (Table XI) followed the same trend as the non-differential segments.

5. Various Treatments.

In all the experiments already reported a temperature of 3°C. was used to produce differentiation in the chromosomes. Various other manipulations of the environment, both physical and chemical, were also used in an attempt to produce differentiation or other chromosomal changes which might furnish further information concerning the phenomenon of differential reactivity. Many of these experiments were performed not with whole plants but with excised roots, which remained healthy and continued to grow for many days.

Physical. Purely physical treatments were of four types:- manipulation of temperature, light, pressure and water content.

(a) Temperature. After 2 days at 35°C. the chromosomes of T. erectum were mostly clumped at metaphase, and no differentiation was observed. They appeared slightly contracted, but the clumping made measurements impossible. A few differential regions were observed in the chromosomes of T. erectum at temperatures up to 13°C. In

T. grandiflorum, however, no differentiation was observed even after 48 hours at 6°C.

(b) Light. Since growth is slower both at low temperatures and under intense illumination it seemed possible that light might also cause differentiation. Excised roots of T. erectum were, therefore, kept in a moist chamber under intense illumination. In one experiment the temperature ranged from 25° to 30°C., and after 24 hours the chromosomes appeared to be slightly contracted, but no differentiation was observed. No metaphases could be measured. After 48 hours no mitoses could be found. At 14° to 22°C. no differentiation was seen after 24 or 48 hours, and at both times the chromosomes appeared slightly contracted. One nucleus from the 24 hour material and one from the control could be measured, and had lengths of 221μ and 322μ respectively. However, no conclusions can be based on these single measurements, particularly as the chromosomes of the control also appeared to be contracted after 48 hours.

(c) Pressure. Excised roots of T. erectum were kept at much reduced pressure for 24 and 48 hours. Slight contraction of the chromosomes and no differentiation were observed after 24 hours. The 48 hour treatment was apparently too drastic. Little change could be seen in the chromosomes after 24 hours at increased pressure.

(d) Water content. One hour in dry air was found to be too severe treatment, and the root-tip cells became

gummy. In boiled distilled water the chromosomes appeared quite normal after 1 hour, but after 24 hours they were clumped and fused, and not readily stainable.

Chemical. The chemical treatments included pH changes, the use of anaesthetics, growth promoting substances, chloral hydrate, acenaphthene and colchicine, the last two also at low temperatures.

(a) Acid. A 5% aqueous solution and fumes from 45% and glacial acetic acid were used. Roots were placed directly in the 5% solution, or in a moist chamber which contained some cotton saturated with 45% or glacial acid. After 1 hour in the 5% solution the chromosomes appeared swollen, and showed very slight indications of differentiation, but not clear enough to be certain. Essentially the same results were observed after 1 and 2 hours in fumes of glacial and 3, 5, and 16 hours in those of 45% acid.

(b) Base. It has been reported (Oura, 1936) that alkaline solutions can unravel the spirals of the chromonemata. Since all cases of marked differentiation of the chromosomes were also accompanied by contraction it was decided to try an agent which might have the opposite effect. Fumes from 0.01N NH_4OH caused clumping of the chromosomes, however, and no other effects could be seen.

(c) Ether. The ether was used in the same manner as the acid and alkali fumes. Plants were placed in a

moist chamber in which there was some cotton saturated with ether. After 1 hour there was no obvious effect, but by 3 hours the chromosomes appeared slightly shortened. No clear separation of the chromatids could be seen at metaphase, and the whole appeared "foggy". Ether was also used mixed with equal parts of mineral oil to reduce evaporation. After 6 hours the chromosomes appeared about their normal length, but took very little stain. After these ether treatments globules of chromatic material were observed in the cytoplasm around the periphery of, and attached to the nucleus.

(d) Growth substances. Both a-naphthyl acetic acid and indolyl butyric acid have been reported to promote root growth. These compounds are nearly insoluble in water, so to obtain greater than physiological concentrations crystals were applied directly to the roots. After 1 hour the chromosomes were clumped, and no other effects could be determined.

(e) Chloral hydrate. Secondary constrictions have been reported by several workers in the chromosomes of a number of genera after treatment with chloral hydrate. Excised roots of T. erectum were kept for 1/4, 1/2 and 1 hour in a 1% solution of chloral hydrate, washed well and placed in a moist chamber for 4 hours before fixation. No differentiation was obtained with any of these treatments.

After 1/4 hour in chloral hydrate the chromosomes were not over-contracted; measurements of five nuclei gave a mean length for the complement of 295 μ . Slight contraction was observed after 1/2 hour in the solution. The mean complement length, for 15 nuclei, was 268 μ . This, however, was not statistically different from the length of untreated chromosomes.

(f) Acenaphthene. The technique employed was the same as in the case of the growth substances. Crystals of acenaphthene were applied directly to the root of T. erectum plants, which were kept in moist chambers. After 24 hours treatment (at 22°C.) the mean length of the chromosome complement was only 216 μ (cf. Swanson, 1940, in Tradescantia). This corresponds with the length after 168 hours at 3°C. In spite of this contraction no differentiation was observed. Some slight alteration in the relative arm lengths resulted (see Table XV), namely a very slight shortening of the B short arm relative to untreated chromosomes.

It seemed probable, from observations on a number of slides, that acenaphthene stopped mitosis at metaphase without inhibiting the initiation of division. Roots were treated with acenaphthene for 96 hours at 3°C., therefore, to see what effect it had on differentiation. Not all the metaphases observed should have passed through a whole

Table XV

Chromosome and chromosome arm lengths, after various treatments, expressed as percentages of the corresponding C chromosome long arms.

Treatment and % standard	A chromosome	B Long arm	B Short arm	C Short arm	C Long arm	D Short arm	E chromosome	Total Length μ
T. erectum								
Chloral hyd.1/4 hr. (0%)	183	156	56	88	133	94	306	295 ±9.7
Chloral hyd.1/2 hr. (0%)	196	171	64	84	128	101	338	268 ±10.5
Acenaphthene 24 hrs. (0%)	188	163	61	81	131	98	322	216 ±6.6
Acen.96 hrs.3°C. (39%)	198	170	77	88	131	100	322	234 ±4.8
Colchicine 24,48 hrs. (0%)	186	165	63	82	133	96	326	194 ±5.9
Colch.96 hrs. 3°C. (27%)	192	169	71	86	133	100	328	231 ±6.4
T. grandiflorum								
Colch.24 hrs. 3°C.24 hrs. (16%)	173	140	61	69	112	85	276	179 ±7.5
Colch.24hrs.3°C. (5%)	165	142	69	76	123	72	290	181 ±6.9

Note: Compare with untreated and cold treatment chromosomes, Table XIII.

division at the low temperature. The mean length of the chromosome complement after this treatment was 234 μ , the same as at the low temperature alone for the same time. Only 39% of the standard regions were observed instead of the 50% for cold treatment alone. Out of the 30 metaphases studied 5 contained 21% standard regions, or less. These might possibly have been at or near metaphase when the treatment was started. The length of the chromosomes, however, suggests that acenaphthene might have had little effect at 3°C.

Roots of T. grandiflorum were also given acenaphthene treatment for 48 and 96 hours at 3°C. The mean percentages of standard regions were 37% and 54% respectively. These values are noticeably lower than those obtained after cold treatment alone (61% and 79% standard). There were no very marked differences between unclei in the number of differential regions after the same treatment. The acenaphthene, therefore, appears to have had the general effect of reducing differentiation in most nuclei. No length measurements were obtained.

(g) Colchicine. This alkaloid has been reported to cause supercontraction of the metaphase chromosomes, and to arrest mitosis at metaphase. Since either of these might be expected to affect differentiation roots of two Trillium species were treated with colchicine solutions, both at room temperatures and at 3°C. In all experiments

a solution of 0.05% was used. T. erectum roots were kept 24 and 48 hours in colchicine solution at 22°C. No difference could be seen in the condition of the chromosomes after these different times, so they were analysed together (see Table XV). The mean length of the chromosome complement was 194 μ, slightly higher than the minimum length obtained by cold treatment (Plate II fig. 10). The chromosomes were not differentiated, and the relative arm lengths differed little from those of untreated chromosomes (cf. Table XIII). As was the case with both acenaphthene and chloral hydrate treatment, there appeared to be a slight tendency for the B chromosome short arm to be overcontracted and the C short arm to be undercontracted, relative to the C long arm.

The chromosomes of roots kept 96 hours in 0.05% colchicine solution at 3°C. had a mean length of 231 per nucleus, almost identical with that after the cold treatment alone. However, the number of differential regions was considerably less with the colchicine -- 27% standard instead of 50%. This parallels closely the results of the similar experiment with acenaphthene. In spite of the moderately high amount of differentiation, the length of the B short arm was almost identical with that of untreated material, relative to their respective C long arms (see Tables XV and XIII).

Two experiments were performed with T. grandiflorum, using both colchicine and cold, but unfortunately data could only be obtained from one plant of each. In the first, roots after 24 hours in 0.05% colchicine solution at room temperature were kept another 24 hours at 3°C. in water. The chromosomes were highly contracted, the mean length of the complement being only 179 μ . Just 16% of the standard regions were differentiated, as compared with 34% for 24 hours at 3°C. with no pretreatment. Although more than half the B short arms contained differential regions, the arm was no longer, relative to the C long arm, than in untreated chromosomes. In the second experiment the roots were kept for 24 hours in the colchicine solution at 3°C. The total length, 181 μ , was almost the same as in the previous experiment. Even fewer differential regions were observed, in fact only 5% standard. With only one B chromosome differentiated in the five nuclei which could be measured, the short arm was relatively longer than in the previous experiment.

It is apparent from the foregoing series of experiments that both acenaphthene and colchicine reduce the amount of differentiation which will be produced by a given cold treatment. They may also cause slight differences in the degree of contraction of the chromosome arms.

6. Chromocentres in the Resting Nucleus.

Counts were made of the chromatic bodies in the

resting nuclei of diploid and haploid tissues of Trillium and other genera, in the hope of discovering whether these really were, as Darlington and LaCour (1940) assumed, identifiable with the differential regions of the metaphase chromosomes. The mean numbers of chromocentres and the range in number are given in Table XVI. The number of these bodies appeared to be the same, in both T. erectum and T. grandiflorum, before and after cold treatment. They showed no response to low temperature such as that shown by the differential regions. Chromocentres were also seen in Tradescantia, Allium, Fritillaria, and Aloe, two of which showed no differential reaction of the chromosomes to cold (see section 7, following).

A comparison of the number of chromocentres in diploid and haploid tissues gave equivocal results, in Trillium erectum and Tradescantia reflexa diploid nuclei, whether root tip or anther wall, contained approximately twice as many of these bodies as did the haploid nuclei of the pollen grains. This would suggest that at least they were directly connected with the chromosomes. However, in Trillium grandiflorum and T. undulatum no such relationship was found; instead there were approximately equal numbers in haploid and diploid nuclei. This might be accounted for by assuming a fusion in diploid but not in haploid nuclei. A 2:1 size relationship of the chromocentres would be expected in that case, however, and

Table XVI

The number of chromatic bodies in the resting nuclei of diploid and haploid tissues

Species and slide	Tissue	No. of Chromatic bodies	Range in number	No. of Nuclei
Trillium erectum				
66-T-1	Root tip (2n)	12.8	7-18	20
72-50-E(3°C)	" " "	13.6	7-24	100
72-90-E(3°C)	" " "	12.4	7-23	100
NW-4E3-4	Anther wall"	11.5±0.38	7-17	50
	Pollen grain (n)	5.3 ±0.24	1- 9	50
66-T-62E	" "	7.6 ±0.33	5-11	20
T. grandiflorum				
72-G-A-50(3°C)	Root tip (2n)	7.8	3-13	50
69-G	" " "	7.4	3-14	20
69-G-149	" " "	6.6	4-14	10
69-G-92	Anther wall "	7.5	5-10	50
	Pollen grain(n)	8.8	4-14	50
T. undulatum				
70-U-16	Anther wall(2n)	11.5±1.41	5-19	50
	Pollen grain(n)	8.2±0.49	5-11	60
Fritillaria meleagris	Root tip (2n)	29.3	18-52	50
Tradescantia reflexa	Anther wall(2n)	5.9 ±0.18	5-7	10
	Pollen grain(n)	2.8 ±0.30	0-5	50

although no measurements were made they appeared to be much the same size in both types of nuclei.

7. Other Genera.

Attempts were made to produce differentiation in the chromosomes of other genera by low temperature. Among the Liliaceae four species of Allium were used. Plants were kept at 3°C. for as long as 336 hours, and roots examined after various intermediate times. No indications of differentiation were seen in any of the chromosomes. Representatives of two other plant families, Tradescantia reflexa Raf. (Commelinaceae) and Vicia faba L. (Leguminosae) also showed no differentiation after 24, 48 and 72 hours at 3°C. However, some differentiation of the chromosomes of Secale cereale L. (Gramineae) was induced by the low temperature. After 72 hours at 3°C. both varieties of rye used (vars. Horton and Rosen) showed a few differential regions of the type seen in Trillium (see Plate III fig.14). No length measurements of untreated rye chromosomes were made, but it was evident that even 24 hours at 3°C. caused quite a marked shortening. The mean lengths of the metaphase complement (14 chromosomes) after 24 and 48 hours at 3°C. were 100 ± 3.7 μ and 67 ± 2.6 μ respectively. The overcontraction occurred much more rapidly than in Trillium, possibly as the result of a higher mitotic rate. Although most chromosomes were very highly contracted after 72 hours

at 3°C., the clearest differential regions were seen in those nuclei where the contraction was not so extreme, and most nuclei showed no differential regions.

Discussion

1. Morphological Differentiation.

Chromosome segments exhibiting differential reactivity are smaller in diameter than the rest of the chromosome and do not stain as darkly, either with aceto-carmine or by the Feulgen technique. From this last fact it may be assumed that they contain a reduced amount of thymonucleic acid. Darlington and La Cour (1940) have explained this as being the result of the "nucleic acid starvation" of the cells. Insufficient nucleic acid in the cell does not allow all parts of the chromosomes to take up the usual amount, and those parts with the lowest affinity for it consequently become differentiated. The work of Caspersson *et al.* has shown, however, that the chromosomes actually synthesise nucleic acid during prophase, rather than take it up from the surrounding medium. Darlington and La Cour's hypothesis must, therefore, be changed at least to "starvation" of some precursor of nucleic acid. Use of Caspersson's ultra-violet absorption method would indicate whether or not there was a smaller than normal amount of pyrimidine containing substances in the cell.

In the present investigation it was found that the differential regions which appeared after only 12 or 24 hours in the cold stained much more darkly than those

seen in metaphases after 4 to 10 days at 3°C. This difference was also observed by Callan (1942) in Triton. These early differential regions appear very similar to segments of prophase chromosomes. On the basis of these observations it is suggested that low temperatures (below about 13°C. in the case of Trillium erectum) reduce or prevent the synthesis of thymonucleic acid by the differential regions during prophase. If a certain cell were at mid-prophase when cold treatment was started, therefore, the differential regions would contain at metaphase only as much thymonucleic acid as they contained at mid-prophase. If this is considered to be the primary cause of differentiation it must be assumed that normal contraction cannot occur in the absence of the normal amount of nucleic acid.

If differential reactivity is fundamentally the prevention of nucleic acid synthesis by the prophase chromosomes, then it should not be possible to produce differentiation in chromosomes which were already at metaphase. Since both acenaphthene and colchicine hold the chromosomes at metaphase, these two substances were used together with low temperature to test this possibility. It would be expected, if no differentiation were produced at metaphase, that certain nuclei which were at that stage when cold treatment was started would show no differentiation. Those which went through division from resting stage

or prophase to metaphase, however, should certain differential regions. The results of the experiments showed no such effect, but instead a general reduction in the degree of differentiation of all nuclei. The question of whether differentiation is possible at metaphase still remains unanswered, therefore, until more critical experiments can be performed.

Darlington and LaCour identified the differential regions observed at metaphase with the chromocentres in the resting nucleus, and suggested that these parts of the chromosomes exhibited "allocycly", being overcharged with nucleic acid in the resting stage and undercharged at metaphase at low temperatures. If these chromocentres actually are the differential regions, then the suggestion that differentiation is caused by the prevention of nucleic acid snythesis during prophase would be invalidated, since it should never be possible to produce the almost achromatic region sometimes seen at metaphase.

No absolute proof was obtained in the present investigation that chromocentres and differential regions are or are not identical. Most of the evidence, however, suggests that they are not. In Trillium erectum and Tradescantia reflexa diploid nuclei contained twice as many chromocentres as haploid ones, but in Trillium grandiflorum and T. undulatum approximately equal numbers were

found in the two types of tissue. Nor was any size difference seen in the last two such as would result from fusion of chromocentres in the diploid nuclei. Also, no correspondence was found between the number of chromocentres and the number of differential regions. For example, anther wall nuclei of both T. erectum, with 42 possible differential regions, and T. undulatum, with 12, contained a mean of 11.5 chromocentres. With no real evidence for the identity of chromocentres and differential regions, the possibility will not be considered further at this time.

The chromonemata appeared to be coiled in most differential regions. Clear enough pictures were never obtained, however, to permit a comparison of the coils in differentiated and non-differentiated parts. If the differential regions actually are in a kind of arrested prophase condition, however, they may be supposed to have relatively more gyres than the non-differentiated parts (see Sparrow, 1942 -- comparison of prophase and metaphase somatic spirals). This would be similar to the condition found by White (1940) in the negatively heteropycnotic sex chromosomes of certain Orthoptera.

Occasionally certain differential regions were seen in which the chromonemata were not coiled. Only short interstitial regions with large blocks of non-differential material on either side of them were found in this condition. It has therefore been assumed that a coil had been formed in these

regions, and subsequently stretched out, either as the result of chromosome movement, or when the material was squashed in the preparation of the slide. It is probable that this is also the explanation of the lack of coiling seen by Callan (1942) in the differential regions of first metaphase bivalents of Triton when these were stretched on the spindle. This is further supported by Callan's statement that in unstretched bivalents the differential regions appeared simply as creases across the chromosomes, like the constrictions between successive gyres of the major spiral. It is, therefore, unnecessary to suppose, as Callan did, that the regions never coiled, as the result of a lack of nucleic acid following a diffuse pachytene with no chromocentres.

Secondary differentiation was frequently observed in the chromosomes of T. erectum. With the single exception of the short arm of the A chromosome, all cases of secondary differentiation involved segments between two differential regions, or between one and the centromere (the centric regions frequently appear sharply differentiated, see Plate II figs. 6 and 7). Secondarily differentiated regions usually showed reduced chromaticity without any noticeable reduction in the diameter of the chromatids (see Plate II fig. 9). The normal diameter of these regions suggests that this is in fact a secondary effect, perhaps a reduction of nucleic acid after metaphase contraction has occurred,

rather than the same type of differentiation that was found in the primary regions. It is tempting to draw an analogy between this situation, in which the region between two differentially reactive segments appears to have a reduced nucleic acid content, and the cases of variegation in Drosophila studied by Caspersson, Schultz and others, where the heterochromatic region affected the nucleic acid content of neighbouring bands of the salivary chromosome. The use of X-rays might show whether the two cases really are analogous. If the appropriate translocations could be produced it should be possible to find whether any part of the chromosome, differential or non-differential in material without translocations, was self-determining with respect to differentiation, or was affected by the neighbouring parts of the chromosome. If differential reactivity is to be of use in taxonomy it is also important to know whether the differential regions are autonomous or not with respect to their reaction.

2. Differential Contraction.

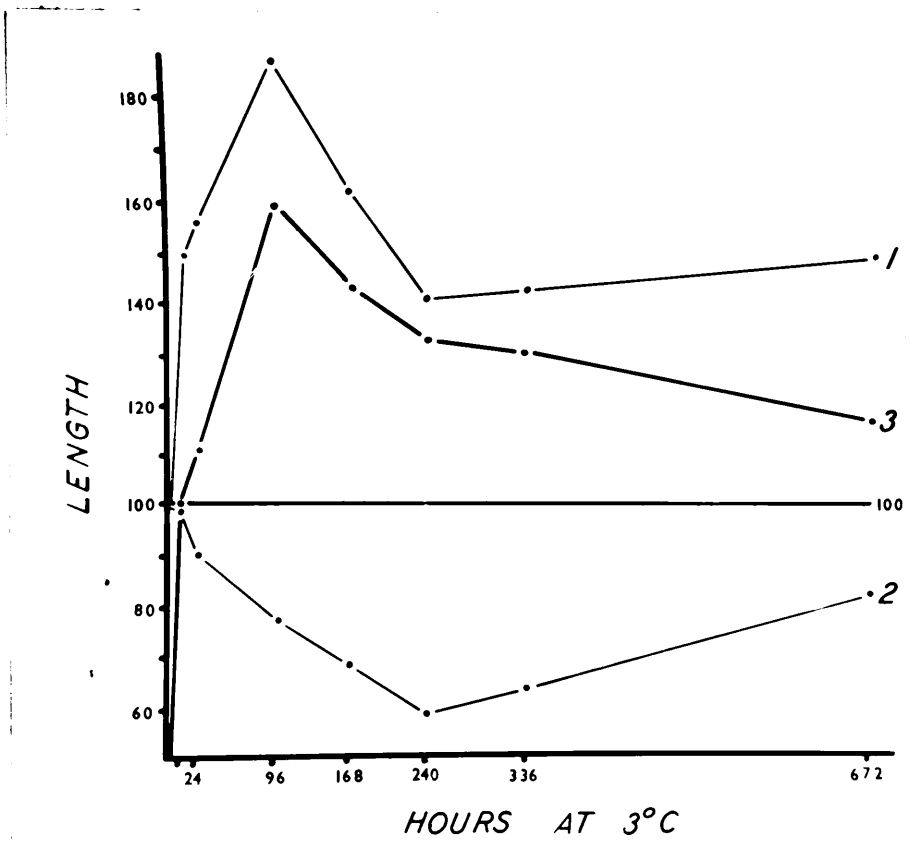
Darlington and La Cour (1938,1940) stated that there was no difference in the length of chromosomes after the same treatment, whether they contained differential regions or not. They also claimed that the differential regions themselves were the same length when showing their special reaction as when not showing it. That is, the length of the

differential segment remained unchanged. No measurements were given to support this assertion. When their camera lucida drawings are measured it is found that differentiated chromosomes of Trillium showed an over-all contraction relative to untreated ones. Chromosome arms with long differential regions were also found from these measurements to be undercontracted, relative to arms from the same material which contained no differential regions. A comparison of the B chromosomes of Paris polyphylla with and without differentiation (Darlington and La Cour,1938, Text-figs 4 and 5) shows that the differentiated ones were shorter than the non-differentiated. The ratio of the length of the arm containing the differentially reactive segment to that without was also greater in the former (1.61 as compared with 1.30 in the chromosomes without differentiation). From these measurements of their drawings it is apparent that in Darlington and La Cour's material, as in that studied here, highly differentiated segments were undercontracted relative to non-differentiated parts of the chromosomes. It must therefore be supposed that the statements of these authors concerning the length of differential segments were not descriptions of their actual findings, but conjectures, which the measurement of a few chromosomes would have shown did not fit the facts.

The case of positive and negative heteropycnosis in the X chromosomes of certain Orthoptera, however, appears to be a different situation. White (1940) gives measurements to support his statement that these chromosomes are the same length at diakinesis, when they show positive heteropycnosis, as they were in the metaphases of early spermatogonial divisions, when negatively heteropycnotic. It must therefore be supposed that negative heteropycnosis in the sex chromosomes of these insects is not the same phenomenon as the differential reaction produced by cold in Trillium chromosomes.

There appears to be no doubt that the differential segments in Trillium are far less contracted than the non-differentiated parts. From Table XIV it is also apparent that the non-differential parts all behave in essentially the same manner. By assuming that the lengths of the non-differential parts of the B and C chromosome short arms of T. erectum bear the same relationship to that of the C long arm in untreated as in cold treated material, it is possible to calculate the length in untreated chromosomes of those terminal segments which become differentiated by cold. That is, it is possible to calculate the length of the differentially reactive regions in the untreated chromosomes. The lengths of the long arm of the C chromosome and of the terminal differential segments of the B and C chromosomes after various durations of cold treatment have been

calculated as percentages of their length in untreated material. These lengths are represented graphically in Text-fig. 13. It will be seen that throughout the cold



Text-fig. 13. Curve 1 represents the length of the B and C terminal differential segments, and curve 2 that of the C chromosome long arm, expressed as percentages of their length in untreated chromosomes. Curve 3 (1-2) shows the difference in contraction of these parts.

treatment the differential parts are always longer and the non-differential shorter than in untreated chromosomes. The interesting feature of this analysis is not these curves, however, but the difference between them. When this difference is plotted (Text-fig. 13, curve 3) it is found that the curve is very similar to that for the number of differential regions observed (Text-fig. 6). The similarity between the two curves is so great that it seems probable that the observation of differential regions depends largely on the degree to which their contraction differs from that of the rest of the chromosome. The difference of their staining reactions is obviously important in this respect, but probably varies directly with contraction. Since the undercontraction of the differential segments is far greater than the overcontraction of the non-differential parts it is probably the more important factor. That visible differentiation is not simply dependent on the condition of the differential regions themselves, but also that of the rest of the chromosome is apparent from a comparison of the condition after 24 and 168 hours at 3°C. After these two treatments the contraction of the differential segments is very similar, but the number of differential regions observed is quite dissimilar.

The fact that it is relative and not absolute contraction which determines the degree of differentiation explains why no direct correlation was found between the

number of differential regions and the over-all contraction of chromosome complement.

An examination of Table XIV brings out an interesting fact. The length changes of differential and of non-differential segments were practically the same after 96 hours at 3°C. whether the material was kept at 3°C. or at 22°C. The main difference was in the timing of the changes. At 22°C., for example, the two types of segment obtained their greatest contraction 3/4 to 1 1/2 hours after the 96 hour cold treatment, while at 3°C. it was not obtained for another five days. Does this mean that that part of the division cycle which at 3°C. takes five days takes only 1 1/2 hour at 22°C.? It does not seem likely that mitosis would be retarded to such an extent at the lower temperature, but no information concerning mitotic rates is available. This lack of information has hampered interpretation throughout the present study. The absolute rates of mitosis at these two temperatures would be very difficult to ascertain. Information concerning relative rates, however, might be obtained from an analysis of chromosome and chromatid breaks at these temperatures following X-radiation.

Darlington and La Cour (1941) stated that " ... in Trillium, nucleic acid starvation requires three days to affect the metaphase of mitosis in the roots. Even at a low temperature this period must comprise most of the resting

stage." They believed that the only metaphases which would show differential regions were those which had undergone the previous resting stage at the low temperature. Our observation of differential regions after only half an hour at 3°C. makes this improbable. It also indicates the importance in interpretation of a knowledge of mitotic rate. Probably half an hour is near the minimum period for the development of a contraction difference great enough to cause visible differentiation.

Knowledge of the length relationships of differential and non-differential parts of the chromosomes provides a tool for the study of variations in differentiation between plants. Darlington and La Cour (1940) found, as has also been found in this present investigation, that pairs of homologous chromosomes within a nucleus did not always show the same differentiation. One or more regions observed in one member of a pair might be missing in another. Also, there might be a difference in the length of certain differential segments between members of a pair of homologues. They stated that this was an indication of the very high degree of hybridity of these Trillium plants, and suggested that the faulty separation of the differential segments which they observed at anaphase in T. stylosum was probably the mechanism by which it was brought about. No such faulty anaphase separation was seen in the four

Trillium species studied here, so it is possible that this is characteristic not of differential segments in general, but simply of those of T. Stylosum.

In T. erectum, the species used for most of the present study, two types of variation were observed, (a) differences in the differential regions observed from cell to cell within plants, and (b) constant differences between plants. As mentioned above, these variations took the form of differences in the length of differential segments, or the complete absence of certain differential regions. On a priori grounds it might be supposed that the first type was caused by minor variations in the environment, or the condition of the cells, possibly the stage of the mitotic cycle which they were at when cold treatment was started. The second type, on the other hand, might be supposed to result from genetic differences between plants. This might take the form either of deficiencies for the segments concerned, or of specific suppression of the differential reaction in those parts.

Variations involving the terminal differential segments of the B and C chromosome short arms lend themselves readily to analysis on the basis of length. All short arms of the B and C chromosomes from material with a high degree of differentiation have been used in this analysis. In this material the lengths of differentiated arms and

undifferentiated arms have been recorded separately as percentages of the corresponding C chromosome long arms. The undifferentiated arms have been further divided into those which never showed differentiation in any cells of the root ("regularly undifferentiated") and those which in other cells of the same root might contain differential regions ("occasionally undifferentiated"). The regularly undifferentiated arms might have a gene-controlled suppression of the reaction, in which case they would be expected to be the same length as the arms of untreated chromosomes, relative to the respective C long arms. They might, however, be deficient for the differentially reactive segment; in that case they should be relatively shorter than untreated arms. In no case should these arms be a higher proportion of the C long arms than are those of untreated roots. Occasionally undifferentiated arms, on the contrary, must be capable of differentiation. It is therefore assumed that they merely had not reached the threshold for visible differentiation. These arms should be at least as high a proportion of the corresponding C long arms as are untreated ones, and in many cases might be expected to be somewhat higher.

In Table XVII are recorded the lengths of the B and C short arms, differentiated, occasionally and regularly undifferentiated, and untreated, and also the non-differential parts of differentiated arms, all expressed as

Table XVII

Lengths of T. erectum B and C Chromosome Short Arms, Differentiated and Undifferentiated, as Percentages of the Corresponding C Long Arm.

Chromosome	Differentiated	Occasionally Undifferentiated	Untreated	Regularly Undifferentiated	Remainder[1]
B	87.4	72.6	69.0	54.1	50.2
C	94.5	83.4	77.0	70.7	68.6

1 Undifferentiated part of differentiated arm.

percentages of the corresponding C chromosome long arms.

As was expected, the occasionally undifferentiated arms were relatively slightly longer than untreated ones, although appreciably shorter than those with differential regions. This indicates that they were not deficient for the differential segments but simply had not attained a visible degree of differentiation. It is contrary to Darlington and La Cour's (1940) explanation of such differences as the result of faulty separation at anaphase.

The regularly undifferentiated arms, on the other hand, were considerably shorter than those of untreated material, relative to their respective C long arms. They were, in fact, practically the same length as the non-differential parts of differentiated arms. It is therefore assumed that their lack of differentiation results from an actual deficiency for the differentially reactive segments. These segments may have been translocated to some other chromosome arm where they are unable to show their special reaction. The other chromosomes, however, appeared unchanged, so it is more probable that the plants were actually deficient for these segments. If this is so, then it is strong evidence in favour of Darlington and La Cour's hypothesis that the differential segments are genetically inert. Several plants were apparently heterozygous for such deficiencies, and one, plant 72-G4, was apparently homozygous for a deficiency of the terminal differential region of the C chromosome.

3. Distribution.

Each of the four Trillium species studied showed a characteristic distribution of differential regions within its chromosomes. Within the species this distribution appeared to be very constant, indicating that translocations were rare. The only types of variation found within a species were deficiencies for certain differential regions in some plants, and variations in the susceptibility of the individual regions to cold.

A comparison of the four species shows certain general areas in which differential regions most frequently occur. These are the third of the A chromosome long arm proximal to the centromere, the distal part of the short arm of the B chromosome, and the short arm of the C chromosome. In addition to these areas the proximal parts of the B and D chromosome long arms and both arms of the E showed differentiation in two of the four species.

It was pointed out in the observations that the distribution of differential regions was somewhat similar in T. erectum and T. cernuum. No differential region was found in the latter which did not have a counterpart in the former. Thus in the A chromosomes regions 1 and 2 in T. cernuum correspond with regions 2 and 3 of T. erectum. In the B chromosomes region 1 of the former corresponds with

region 2 of the latter, although slightly closer to the centromere. The indentical situation exists in the C chromosomes, where region 1 corresponds with region 2, while in the E chromosomes 1 and 1a are corresponding regions in the two species. The most striking difference between the two is the absence in T. cernuum of the long terminal differential segments in the short arms of the B and C chromosomes of T. erectum. Both these arms are very short in the former species, and the finding of a T. erectum plant with a homozygous deficiency for one pair suggests that T.cernuum is deficient for both these segments found in T. erectum.

It is possible that the study of differential regions might be useful for the solution of certain taxonomic problems. For example, Trillium erectum var. album has been suggested by some workers to be a natural hybrid between T. erectum and T. grandiflorum. The examination of differential regions in its chromosomes might answer this question quite simply. If it is merely a colour variant of T. erectum, then it should show the distribution of differential regions typical for that species. If, on the other hand, it is a hybrid there are several possibilities as to the type of differentiation which would be found. The chromosomes might remain self-determining, in which case typical chromosomes of each species should be found. On the other hand, the changed genotype might change the expression

of the differential reaction, or even suppress it.

Differential reactivity might also be used to determine the presence or absence of apomixis in Trillium. It has already been found that T. erectum plants will set seed when pollinated by T. grandiflorum. The presence or absence of embryos in these seed has not been investigated here. If embryos are found, however, it should be possible, by inducing differentiation, to determine whether they are hybrid or purely maternal.

Summary

(1) Regions of the mitotic chromosomes which show a differential reaction to low temperatures have been studied in four species of Trillium. At room temperature these segments were morphologically indistinguishable from the rest of the chromosome. At low temperatures, however, they became visibly differentiated from late prophase to telophase.

(2) When root tips grown at low temperature (3°C) were fixed in 3:1 alcohol-acetic acid, and examined without staining, the differential regions could be seen at metaphase as segments in which the chromatids had a reduced diameter. When such material was stained either with aceto-carmine or by the Feulgen technique the differential segments did not stain as darkly as did the rest of the chromosome.

(3) The chromonemata appeared to be coiled in the differential regions, but no comparison of this coil with that in non-differential parts was possible. In certain instances the chromonemata appeared uncoiled in differential segments. This has been explained as the result of stretching, rather than the inherent inability of the regions to form spirals.

(4) Each of the four Trillium species studied had a constant and characteristic distribution of differential regions in its chromosomes. T. erectum had 42 potential

differential regions per mitotic metaphase complement, while 12 possible regions were found in each of the other three species, T. cernuum, T. grandiflorum and T. undulatum. No differential segment was found in T. cernuum which did not have a counterpart in T. erectum. From the length of its chromosome arms the former species appeared to be deficient for the two longest differential regions of the latter.

(5) At 3°C. the first differential regions were seen in T. erectum chromosomes after 1/2 hour. The number of differentiated regions per nucleus increased with cold treatment to a maximum, after 96 hours, of 40% of the potential regions. Longer cold treatment resulted in a reduction of the number of visibly differentiated segments per nucleus. Essentially the same results were obtained with T. grandiflorum.

(6) When plants which had undergone cold treatment were returned to room temperature, or higher, the number of visibly differentiated regions per metaphase complement rapidly decreased. Twelve to 24 hours after cessation of cold treatment nearly all signs of differentiation had disappeared.

(7) Differences were found, both within and between plants, in the differential segments which were observed. Some of these differences appeared to be merely the result of fortuitous variation, while others were constant and must be assumedcto be the result of genetic differences

between the plants.

(8) Secondary differentiation was also observed. This showed as understaining of the usually non-differential parts between differential segments, without, however, any appreciable reduction in their diameter.

(9) In cold treated chromosomes the differential segments were undercontracted and the non-differential parts overcontracted, relative to untreated chromosomes. The amount of differentiation seen at any time appeared to be a function of the difference in the degree of contraction of differential and non-differential parts.

(10) The use of various other environmental agencies, both physical and chemical, failed to cause differentiation, although certain of them produced chromosome contraction.

(11) Most of the evidence suggested that the chromocentres of the resting nucleus were not the same as the differential segments seen at metaphase.

(12) Of four other genera given cold treatment, Allium, Tradescantia, Vicia and Secale, only the last-mentioned showed any differential segments.

(13) Certain plants were found which had a homozygous deficiency for certain differential regions. This has been taken as evidence that these segments are probably less active genetically than the non-differential parts.

(14) The inhibition of nucleic acid synthesis in the differential regions during prophase has been suggested as a possible mode of action of low temperature in the pro-

duction of differentiation. The reduced contraction of differential regions might be the result of the lack of nucleic acid synthesis. It might, however, be independently caused by the low temperature, or both effects might be the result of some single earlier action.

(15) Certain further experiments are suggested which might give more information on the nature of the differential reaction.

(16) The application of a study of differential reactivity to the solution of certain other problems has been suggested.

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Description of Plates

Plate I

Magnification 1550x. Nuclei of T. erectum after 96 hours at 3°C.

Fig. 1. Root-tip metaphase showing differential regions. Note the stretched differential segments in one D and one E chromosome, and also the slight indication of the separation of differential regions 1 and 4 in one B chromosome.

Fig. 2. Anaphase from the same root tip as fig. 1.

Plate II

Magnification 900x. Nuclei of T. erectum. Figs. 3 to 8 metaphases showing changes in the degree of contraction and differentiation of the chromosomes during cold treatment.

Fig. 3. Untreated.
Fig. 4. 12 hours at 3°C.
Fig. 5. 24 hours at 3°C.
Fig. 6. 96 hours at 3°C. Greatest differentiation.
Fig. 7. 240 hours at 3°C. Greatest contraction.
Fig. 8. 672 hours at 3°C.
Fig. 9. 240 hours at 3°C. A and E chromosomes show clear cases of "secondary differentiation."
Fig. 10. Metaphase after 48 hours in 0.05% colchicine solution. Chromosomes contracted.

Plate III

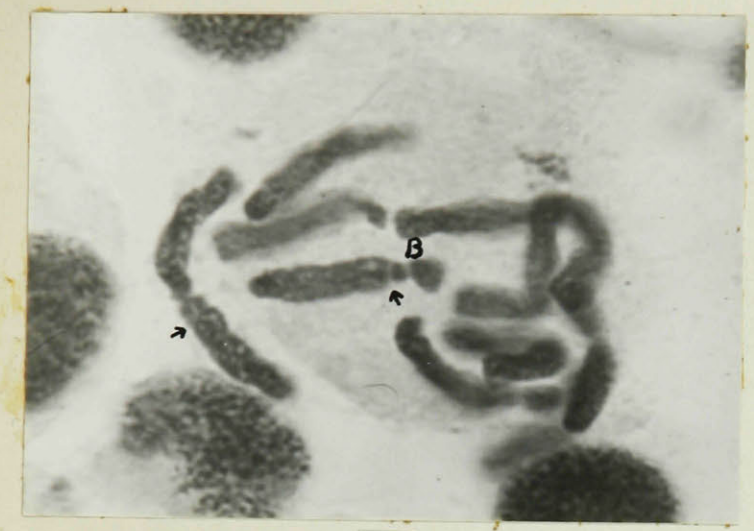
Magnification: *Trillium* 900x, *Secale* 1250x.

Fig. 11. *T. cernuum*, 96 hours at 3°C.
Fig. 12. *T. grandiflorum*, 48 hours at 3°C.
Fig. 13. *T. undulatum*, 96 hours at 3°C.
Fig. 14. *S. cereale*, 72 hours at 3°C.
Fig. 15. *S. cereale*, 72 hours at 3°C.
Fig. 16. *T. erectum*, 3/4 hour at 22°C. after 96 hours at 3°C. Chromosomes highly contracted but undifferentiated.

PLATE I

PLATE II

PLATE III



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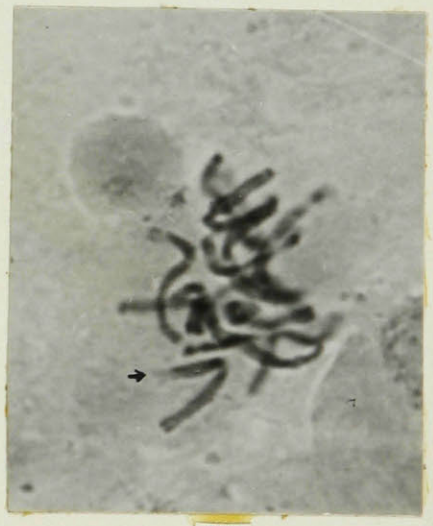

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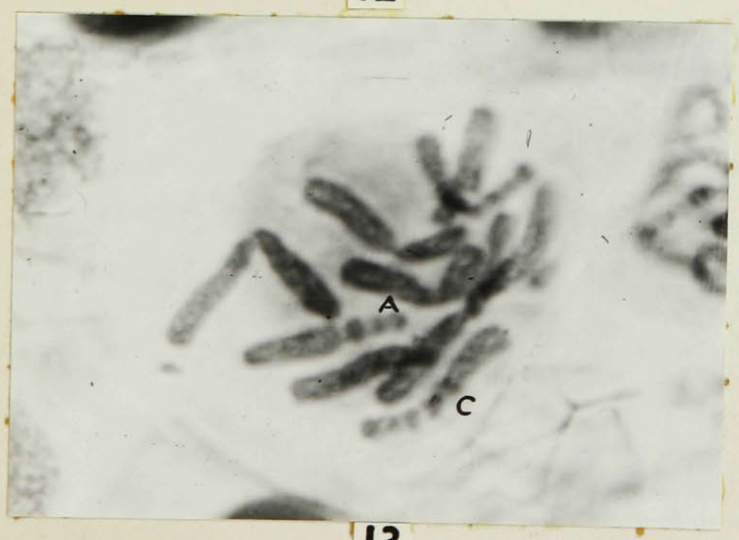
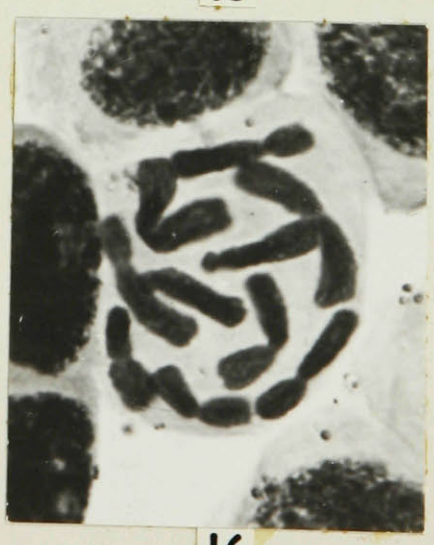


13



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