STUDIES OF A TELOCENTRIC CHROMOSOME IN MAIZE WITH REFERENCE TO THE STABILITY OF ITS CENTROMERE

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INTRODUCTION

EVERY chromosome possesses a differentiated region which has been designated by a variety of terms such as 'kinetochore,' 'spindle attachment region,' 'insertion region' and 'centromere.' Beginning with METZNER (1894) it has been believed that this differentiated region, which we shall call the centromere following DARLINGTON, played an essential rôle in chromosome dynamics, being involved in the congression of the chromosomes to the metaphase plate and in their poleward movements at anaphase. Conclusive proof of these probable functions, however, was obtained from the mitotic behavior of acentric fragments which arise through crossing over in heterozygous inversions or by breakage of the chromonemata, and are found to lie as passive bodies, or behave irregularly, upon the acromatic figure.

As is well known it is usually the centromere which leads the way in the anaphase movement of the chromosome towards the pole but there are a few notable exceptions. For example, in the monocentric spindles in Sciara (METZ 1938) one group of chromosomes passes away from the pole even though they show by their orientation, shape, and proximal attenuation that a force located at the centromere is being exerted upon them to move in the direction of the pole. METZ believes that the unusual behavior in these monocentric mitoses indicates the presence of two distinct forces governing chromosome movement. One of these is centered at the centromere while the other appears to be distributed through the length of the chromosome and not centered at one point. However, in the case of bipolar mitoses it is the movement controlled by the centromere which is recognizable and the second force while present and operating cannot be distinguished because of the nature of the bipolar spindle. METZ'S observations and BELAR'S (1928) studies with living spindles, in which he found evidence that a portion of the anaphase movement of the chromosomes was due to the expansion of the middle part of the spindle thus pushing the chromosomes apart, make it clear that the centromere is not the sole agent concerned in anaphase movement. That it is an essential one there is no doubt.

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In recent years a number of investigators, especially SCHRADER (1932, 1936, 1939) and TRANKOWSKY (1930), have become concerned with the structure of the centromere. SCHRADER describes the centromere in the amphibian Amphiuma as a compound body composed of a commissural cup enclosing a minute chromatic spherule which is directly involved in the formation of the half-spindle fiber. The chromatic spherules of SCHRADER are analogous to the kinetic bodies described by SHARP (1934). It may be questioned, however, if the structure of the centromere is identical in all organisms. In maize, where in aceto-carmine smears the centromeres of the paired chromosomes at pachytene have a homogeneous, translucent appearance, the writer has never seen convincing evidence of the presence of chromatic spherules nor were they apparent at meiotic metaphase and anaphase where they should be readily observed (see figure A, Plate 2 and figures A and B, Plate 3). SCHRADER (1939) suggests that the centromeres of Amphiuma and Zea are basically alike in structure and that the apparent dissimilarity between them at meiotic anaphase results from differences in resistance to mitotic separation. However, SCHRADER finds evidence of structural heterogeneity in the centromeres of Amphiuma chromosomes in the late meiotic prophases—a condition which has not been observed in the centric regions of Zea chromosomes, although, it must be admitted, different fixing and staining methods might disclose a more complex structure.

The centric regions of both Zea and Amphiuma exhibit staining reactions which differ from those of the rest of the chromosome. SCHRADER noted that the centrosomes and the chromatic spherules have similar staining properties and suggested a relationship between these two bodies. POLLISTER (1939) reported a striking correlation between the numbers of centrioles and centromeres in Vivapara where it appears that the centromeres may become disassociated from degenerating chromosomes and take on the properties of centrioles. Unfortunately, in maize the presence of clearly defined centrosomes and chromatic spherules has never been established. Even though the structure of the centric region may not be identical in all organisms, it is apparent that it is a well differentiated region of the chromosome which has a specialized function quite distinct from that of any chromomere.

The centromere is not concerned solely with chromosome movement. It divides the chromosome into two arms which behave in some respects as independent units since the direction of relational coiling in the two arms is at random (SAX 1936) and the genetic phenomenon of interference does not extend from one arm to the other across the centromere. It has also been established, in Drosophila particularly, that the frequency of crossing over is reduced in those regions adjacent to the centromere.

MATHER (1936) has suggested that this reduction in crossing over in the proximal regions may account for their genetical inertness and the accompanying accumulation of heterochromatin. UPCOTT (1937) believes that cell wall formation occurs under centric control. DARLINGTON (1937) believes that the centric region of the chromosome effects the structure of the spindle.

This list of suggested functions of the centromere makes no claim to completeness but it does illustrate that the centromere plays a variety of rôles in mitosis.

The terms telomitic and telocentric have been used to describe chromosomes with an apparently terminal centromere while the terms atelomitic and atelocentric indicate that the centromere is non-terminal. A number of investigators, especially S. NAWASCHIN (1916) and LEWITSKY (1931) who have stated their position clearly and unequivocally, hold that no chromosome has a true terminal centromere. It is maintained that those chromosomes which ostensibly have a single arm possess a minute second arm so small as to escape observation unless special techniques are used at critical stages. That this view may be correct is indicated by the demonstration that a number of rod-shaped chromosomes long believed to be telocentric actually have a sub-terminal and not a terminal centromere. A particularly impressive investigation has recently been made with the minute fourth chromosome of *Drosophila melanogaster* which is so small that it has a dot-like shape in oogonial cells with no indication of being other than telocentric. However, KAUFMANN (1934) from his study of the somatic prophases believed the fourth chromosome to be two-armed. Later, GRIFFEN and STONE (1939) obtained genetic and cytological evidence confirmatory of KAUFMANN'S observation. KAUFMANN also showed that the rod-shaped X chromosome of *melanogaster* has a sub-terminal centromere. The rod-shaped chromosomes of certain Orthoptera have long been held to possess terminal centromeres. DARLINGTON (1936) however states that none of the chromosomes of Chorthippus and Stauroderus possesses a terminal centromere. The joining of two rod-shaped chromosomes at the centromere to form a V-shaped element has been observed in certain Orthoptera (KING and BEAMS 1938). While this has been taken to prove that the rod-shaped chromosomes are telocentric, it is not improbable that they are similar to the X of *Drosophila melanogaster* in that they possess a minute short arm composed of genetically inert material. Unequal translocation could result in the two genetically active long arms becoming attached to a common centromere. Whether or not the two short arms were lost or retained would be of no consequence if they are composed of inert material.

There is apparently no certain case of a telocentric chromosome in the

regular chromosomal complement of any plant, and it is possible, though not as thoroughly established, that a similar condition is true among animals. The failure to find a single undoubted case of a telocentric chromosome in the regular complement of any organism suggests that a centromere so placed is either unable to function properly or is unstable (*cf.* DARLINGTON 1939). The writer (1936) described the occurrence and behavior of a supernumerary telocentric chromosome. It would appear, therefore, that the failure to find terminal centromeres in the normal chromosome complement may be due to their instability and that this instability has led to their disappearance through selection. Further study of the telocentric chromosome mentioned above has yielded data which are pertinent to the consideration of the stability of terminal centromeres.

A TELOCENTRIC CHROMOSOME IN MAIZE

Maize has a haploid set of ten chromosomes. MCCLINTOCK (1933) has shown that each member of the complement can be recognized by its architecture. No member of the regular chromosome complement has a terminal centromere. The fifth longest chromosome has been associated with the *a2-bm-Pr-v2* linkage group. This chromosome has its centromere in a nearly median position. The ratio of the length of the two arms is 1.1 : 1.0. In certain strains of maize the longer arm often carries a prominent knob which facilitates distinguishing between the two arms. Plants trisomic for chromosome 5 differ markedly in their appearance from disomic sibs. They have thicker, broader leaves with blunter tips, a stubbier tassel, and a shorter stature than do disomes. There is no difficulty in classifying a segregating progeny into disomic and trisomic types.

In 1933 among the progeny of a plant trisomic for chromosome 5 there occurred a single plant which was intermediate in appearance between its trisomic and disomic sibs. A cytological examination of this exceptional plant disclosed that it possessed 21 chromosomes but that the extra chromosome consisted of the short arm only of chromosome 5. It had a terminal centromere. It arose through a break at or in the centromere of a normal chromosome 5. It is difficult to ascertain if the size of the centromere on the short arm of chromosome 5 is identical with that of a normal chromosome 5 because the apparent size of a centromere varies considerably in different cells. The short arm, however, does possess a readily visible centromere approaching in size that of a normal chromosome 5. While there is no proof that this terminal centromere arose from a fracturing of the parental centromere, it is of interest to note that MCCLINTOCK (1932, 1938) has shown that both parts of a transversely broken centromere are capable of functioning.

Previously (1936) this chromosome was described as a 'fragment' chro-

mosome but since this term has been so widely used to denote acentric chromosomes arising either spontaneously or through irradiation the term 'telocentric' is preferred and will be used to describe it. In the 1936 paper the method was described by which the telocentric chromosome consisting of the short arm of chromosome 5 was utilized in placing the genes of the fifth linkage group in the long and short arms of the chromosome. According to the data summarized by EMERSON, BEADLE and FRASER (1935) the order with intervening crossover values of 6 of the 23 genes in this group is *a2* (6) *bm* (6) *bv* (19) *pr* (9) *ys* (32) *v2*. The available linkage data were insufficient to place accurately the remaining 17 genes. Eight of the genes in the fifth linkage group were tested against the telocentric chromosome and the *v2 ys pr v12 v3* and *bt* loci were found to lie in the long arm and *a2* and *bm* in the short arm of chromosome 5. The data presented in table 1

TABLE 1

Summary of Bm Bt Pr backcross data.

F_1 GENOTYPE	PARENTAL COMBINATIONS	REGION 1	REGION 2	REGIONS 1 AND 2	TOTAL
Bm bt pr / *bm Bt Pr*	135 462	8 3	92 268	2 2	972
		1.13%	37.04%	0.41%	
			Bm–*Bt*=1.5%		
			Bt–*Pr*=37.5%		

The inequality of the complementary classes is due to the poor germination of *bt* seed.

show that the order is *bm bt Pr* and that there is only 1.5 percent of recombination between *bm* and *bt* although they lie on opposite sides of the centromere. MCCLINTOCK (1938) from her study of ring-shaped fragments placed *bm* in the short arm close to the centromere. STADLER (1935) obtained a deficiency in the long arm of chromosome 5 which included the *v3* locus but not the neighboring loci of *bm*, *bt* or *bv*. BURNHAM (1934) reported that the order of genes going from the end of the long arm towards the centromere is *v2 ys pr bv bm*. RHOADES (1933b) from a study of a reciprocal translocation indicated that both *bm* and *bt* were close to the centromere of chromosome 5 and BURNHAM reached the same conclusion from his study of another translocation. The above cited data from various investigators give the same placement of loci arrived at by the use of the telocentric chromosome. Utilizing the cytogenetic data it is possible to place both *bt* and *v3* in the linkage map which becomes:

0	6	8	10	12		31	40		72
a2	*bm*	*bt*	*v3*	*bv*		*pr*	*ys*		*v2*

CYTOLOGICAL STUDIES

Synapsis of the telocentric chromosome with the two normal chromosomes 5 was studied in plants hyperploid for the telocentric chromosome. A number of clear pachytene figures was obtained in which the synaptic relationships could be determined (figure 1). At any given region pairing between the three homologous short arms was always in twos, with the

FIGURE 1.—Camera lucida drawings at pachytene showing synapsis of telocentric chromosome with the two normal chromosomes 5. The centromeres are represented by clear ovals or circles and the prominent knob in the long arm, when present, by dark ellipses.

third arm unpaired. Exchanges of pairing mates among the three arms were not frequent; in the majority of pachytene figures there was a single exchange of partners and the greatest number observed was three. It should be clearly understood that the exchange of pairing mates referred to is between the three homologous arms and should not be taken to indicate the existence of chiasmata. It is, of course, true that the observed exchange of partners must occur before chiasmata can be formed between the different chromosomes, but there is no reason to believe that a chiasma is formed in every paired segment lying between the points where exchanges of partners occur, especially if the paired region is short. In fact,

if the partial chiasmatype theory of crossing over is correct, which seems likely, it is not proper to define a chiasma as consisting of an exchange of the pairing elements (that is, chromatids), since sister chromatids are paired on both sides of a chiasma; all that has occurred is a breakage and reunion of ends between two non-sister chromatids. The term exchange of partners is used correctly to describe the exchange of partners among homologous chromosomes in polyploids or in the case of a reciprocal translocation where structural dissimilarity causes a change of mates.

It is evident from figure 1 that pairing between the telocentric chromosome and a normal chromosome 5 does not necessarily commence at the centromere, as the terminal centromere often lies to one side of the two paired centromeres of the normal chromosomes 5; and in some cases was observed 'stuck' to the centromeres of other pairs of chromosomes without preventing synapsis in distally placed regions.

Plate 2, figure B is a photomicrograph of an unpaired telocentric chromosome at pachytene. The equational division or split of this chromosome into chromatids is evident at the distal end. The terminal centromere, which unfortunately cannot be clearly seen in the photograph, appears to be divided or possibly is beginning to divide inasmuch as its distal end is cleft or heart-shaped. As the writer stated in 1936, this apparent division of the terminal centromere at mid-prophase may or may not be representative of the behavior of paired centromeres interstitially located. In a number of organisms the genetic and cytological evidence is convincing that the first meiotic anaphase is reductional for the centric regions of bivalent chromosomes; irrespective of the physical state of division of the centric region of two sister chromatids it acts as a single functional unit. As figure A, Plate 3 suggests, and indeed as has been reported by both SCHRADER and DARLINGTON, each chromatid of the metaphase tetrad may give rise to its own half-spindle component. But SCHRADER points out that it is the chromatic spherules in Amphiuma which give rise to the half-spindle components, and while each chromatid has its own chromatic spherule the commissural cup in which they both lie has not divided so the disjunction of the sister chromatids at the centric region is necessarily reductional. Figure B, Plate 3 also indicates that the bulk of the centric region has not divided even though it clearly shows that each chromatid will form what SCHRADER terms its half-spindle component.

The frequency of trivalent association at metaphase I between the telocentric and the two normal chromosomes 5 was determined in microsporocytes. Metaphase counts were made in five plants and the mean frequency of trivalent association found to be 59 percent. The frequency of trivalents ranged from 50 to 70 percent in different plants. Whether or not this difference is genetic or caused by environmental factors cannot be stated,

but that it is genetic is suggested by the fact that different anthers of one plant gave consistently higher values than did anthers of another. Genetic data to be reported in a later section on the difference between the frequencies of plants hyperploid for the telocentric chromosome in two strains segregating for disomic and hyperploid individuals also argues for a genetic basis.

Figure E, Plate 2 is a photomicrograph of a trivalent group at metaphase I which is typical of the majority of cases where a trivalent occurs. The telocentric chromosome is oriented on the spindle in such a way that it will pass to a pole with one of the normal chromosomes 5, which will disjoin from each other. This non-random orientation of the trivalent, which leads to non-random distribution, is a natural and logical consequence of the equilibrium position attained by the interaction of the three centromeres, which tend to repel each other, and of the chiasmata by which the association of paired chromosomes is maintained through metaphase. In most cells one chiasma, at least, is formed between the two long arms of the two normal chromosomes and one between their two short arms. If in addition a chiasma is formed between the telocentric chromosome and one or other of the two short arms of the normal chromosomes 5, this latter chiasma comes to occupy a terminal position because of the generalized repulsion existing at this time between chromosomes as bodies, in addition to the localized centromere repulsion (*cf.* DARLINGTON 1937). Since both arms of the two normal chromosomes remain associated by chiasmata they will tend to lie symmetrically upon the spindle with their centric regions oriented towards opposite poles. The telocentric chromosome which is associated distally with the normal chromosomes by a triple terminal chiasma will lie more or less in the longitudinal axis of the spindle (that is, at right angles to the equatorial plate) with its terminal centromere directed poleward. The end result is that the centromeres of the two normal chromosomes are oriented against one another while that of the telocentric chromosome is not subject to such regulation. The type of disjunction is not determined by the centromeres themselves. Their orientation on the spindle is a function of the metaphase configuration produced by prophase pairing and chiasma formation. Such metaphase configurations as were commonly observed would be expected to produce anaphase disjunctions in which the two normal chromosomes 5 disjoin from each other with the telocentric chromosome accompanying one or other of the normals. These configurations rarely should give an anaphase distribution in which the two normals pass to the same pole while the telocentric chromosome goes to the opposite one. The genetic data in tables 2–5 amply confirm this expectation.

When the telocentric chromosome is a member of a trivalent group it passes poleward at the same time at which the bivalents are disjoining. It is recognizable during anaphase I because, possessing a terminal centromere, it has a V-shaped appearance resulting from the attachment of the two chromatids at the undivided centromere while their distal ends are some distance apart. The other dyads in anaphase I have a double V- or double J-shaped appearance, depending upon the relative lengths of their two arms, with the apices of the V's or J's attached to a common centromere.

In prophase II the two monads of the telocentric chromosome, which will separate equationally in the subsequent anaphase, form a single rod-shaped chromosome as they are conjoined by the still undivided centromere. The repulsion between the two chromatids is so pronounced at this stage that they tend to lie in a straight line. The centromere does not appear in its usual position but is forced to one side and the two chromatids appear fused at their proximal ends. This specious appearance is even more clearly illustrated in the case of the other dyads, especially those where the two arms differ greatly in length. Here the two short arms appear joined into a single element while the two long arms also appear united into a single body, the two elements being separated by the centromeric region (figure G, Plate 3). When the equational separation of the two chromatids of the telocentric dyad occurs in anaphase II, the monads (chromatids) appear as rod-shaped bodies with the terminal centromere leading the way to the pole. The other monads appear as single V's or J's, depending upon the location of the centromere (figure J, Plate 2).

The unpaired telocentric chromosome usually lay on the spindle at metaphase I but in some cells failed to move onto the plate (figure G, Plate 2). Its behavior at anaphase was variable. After the bivalents had completed their anaphase movements, its two halves often would separate equationally and the daughter univalents would begin their migrations to opposite poles. That they were able occasionally at least to complete their journey to the poles in time to be included in the interphase nuclei was made evident by the observation of daughter univalents in prophase II. The equational separation of the univalent occurs so much later than the disjunction of the bivalents that the writer previously (1936) believed they failed to divide equationally in the first meiotic division, but this was an erroneous conclusion drawn from the study of too early anaphases. While the univalent usually divided equationally in anaphase I, it did not always do so because it was not uncommon to find the chromosomes at the two poles in interphase with the univalent lying to one side in the cytoplasm. Presumably it was those univalents that congressed which

later divided equationally at anaphase while those that failed to congress become laggards. When they lie near a pole, lagging univalents may in some cases be drawn fortuitously into the telophase nucleus.

When a daughter univalent succeeded in reaching the pole in anaphase I, it lagged in the following anaphase because it had precociously undergone the equational separation which normally occurs in the second division (figures H, I, Plate 2). No detailed counts were made but it seemed that the number of lagging chromosomes at anaphase II was less than twice the number of univalents which split equationally in the first division. UPCOTT (1937) noticed a similar phenomenon in Tulipa and suggested that some of the daughter univalents were carried to the poles in anaphase II along with the dividing chromosomes. Another possibility is that the daughter univalents which do not reach the poles in anaphase I fail to congress at metaphase II and, lying off of the spindle, would not appear to be lagging. This point is worthy of more study, but the cytological observations indicate that the telocentric chromosome was almost invariably lost through lagging at the first or second meiotic divisions when it was unpaired at metaphase I. The genetic data on the frequency of hyperploid individuals in the progeny of a hyperploid plant is the best evidence that the unpaired telocentric chromosome usually suffered elimination. Conversely, the telocentric chromosome underwent normal meiotic behavior when it was a member of a trivalent group. In brief, the cytological observations show that (1) the usual orientation of the trivalent group on the metaphase plate was of such a nature as to lead to a nonrandom distribution in anaphase. The gametes should consist mainly of two types, namely those that are haploid and those hyperploid for the telocentric chromosome, with relatively few gametes having two normal chromosomes 5 or the telocentric chromosome only. (2) The observed frequency of trivalents at metaphase permits the prediction of the relative numbers and types of offspring expected in the progeny of a plant hyperploid for the telocentric chromosome, since the unpaired telocentric is usually eliminated when it is an univalent.

GENETIC STUDIES WITH THE TELOCENTRIC CHROMOSOME

The *a2* and *bm* loci were reported by the writer (1936) to lie in the short arm of chromosome 5. A strain was obtained hyperploid for the telocentric chromosome carrying the recessive *bm* allele in each of the normal chromosomes 5 and the dominant allele in the telocentric chromosome. These hyperploid plants were crossed reciprocally with diploid *bm* individuals and the progeny classified for the *bm* character and the different chromosomal types. Table 2 summarizes the results obtained using the hyperploid individuals both as the male and female parent in backcrosses. The data

show that 98.91 percent of the progeny obtained when hyperploid plants were used as the male are diploids homozygous for the recessive allele *bm*. These arose from the functioning of a haploid pollen grain carrying *bm* in a normal chromosome 5. Eighteen of the 7,245 plants or 0.25 percent were diploids not exhibiting the *bm* character and therefore carried the dominant allele in the chromosome contributed by the hyperploid parent. This type of chromosome arose from a crossover between the telocentric chromosome and a normal chromosome in the *bm*-centromere interval so that the dominant allele was transferred to the normal chromosome. Thirty-seven or 0.51 percent of the offspring were hyperploid for the telocentric chromosome and were identical in constitution with the male parent. These individuals arose through the functioning of a pollen grain carrying the telocentric chromosome with the dominant allele and a normal chromosome 5 with a recessive allele. As the cytological observations show that something over thirty percent of the grains should be hyperploid for the telocentric chromosome, the genetic data show that the hyperploid grains do not successfully compete with haploid pollen and that it is only an occasional hyperploid grain which effects fertilization. Hyperploid pollen grains are well filled with starch and cannot be distinguished in appearance from haploid grains. The ineffectiveness of hyperploid grains in accomplishing fertilization in competition with haploid pollen is probably due to a slower rate of pollen tube growth.

Two *bm* individuals were primary trisomes of chromosome 5. These plants arose from a gamete with two normal chromosomes 5. If they were contributed by the male it follows that two events must have occurred. First, the disjunction in anaphase I must have been such that the two normal chromosomes 5 went to the same pole. That such disjunction occurs is shown by the data in the same table where 1.63 percent of the eggs received two normal chromosomes 5. Second, such a hyperploid pollen grain with two normal chromosomes 5 must have functioned in competition with haploid pollen. That such grains occasionally do compete successfully was shown in the experiment in which related primary trisomes of chromosome 5 were used as the male parent, five plants in the total of 1,212 offspring being primary trisomes. The product of the frequency of the two events gives a probability of less than one such individual expected where two were observed. There is also the possibility that an egg with two chromosomes 5 arose in the diploid female parent through non-disjunction. Such spontaneous occurrences of primary trisomes have been observed but they are so rarely found that nothing is known of their frequency. It seems not unreasonable to suppose that the two primary trisomes arose from a male gamete in the manner suggested.

The foregoing classes with their observed frequencies can be readily ac-

counted for. In addition to them, however, there is a class which is entirely unexpected as it includes a type of chromosome absent in either parent. This class consists of the 22 secondary trisomes which possess a supernumerary chromosome consisting of two short arms of chromosome 5 attached to a single, median centromere. Twenty-one of the secondary trisomes were *Bm* and one was *bm*. This suggested that the telocentric chromosome was involved in the formation of the secondary or iso-chromosome since it carried the *Bm* allele. The exceptional *bm* secondary trisome

FIGURE 3.—The seedling to the left is a secondary trisome in which the secondary chromosome is composed of two short arms of chromosome 5 attached to a median centromere. The secondary chromosome arose through misdivision of the centromere of a telocentric chromosome consisting of the short arm of chromosome 5. The seedling to the right is a sibling of the secondary trisome and is a diploid. The differences in growth and texture of the leaves are quite pronounced between the secondary trisome and its disomic sib.

could be accounted for by a crossover between the telocentric chromosome and a normal chromosome 5. It also is possible that it arose from a normal chromosome 5 by transverse division of the centromere. Data presented in other tables leave no doubt that the telocentric chromosome is involved in the formation of the new type of chromosome. The secondary trisomes were strikingly different in appearance from any of the other chromosomal types. Their dwarf-like habit and thick leaves of leathery texture made them easily recognizable as seedlings (see figure 3) while the other chromosomal types could not be accurately classified until a much later stage.

Hyperploid plants used as female parents in backcrosses with diploid *bm* individuals yielded progenies distinctly different in some respects from those obtained when the hyperploid plants were used as the male parent. As table 2 shows, there were 10 diploid *Bm bm* plants out of a total of 5,523, or a percentage of 0.18. This value agrees very well with the percentage of 0.25 for the same class when the hyperploids were used as the male. These plants originate as before from a crossover between the telocentric chromosome and a normal chromosome 5 in the *bm*-centromere region. There were 3,738 (67.68 percent) diploid *bm* individuals. This class comes from haploid gametes with non-crossover chromosomes. The frequency of this class is much lower than in the reciprocal backcrosses as there were 1,671 or 30.26 percent hyperploid plants while only 0.51 percent of the offspring were hyperploid *Bm* plants when hyperploid individuals were used as the male parent. The difference between the two kinds of backcrosses in the frequency of the hyperploid *Bm* is due to the fact that there is little or no competition between megaspores. If the basal megaspore of the quartet happens to receive the telocentric chromosome in addition to a normal chromosome 5, it develops without competition into a functional embryo sac; but a hyperploid pollen grain never enjoys such a positional advantage and must compete against haploid spores. The data obtained when hyperploid plants were used as female parents also differ from the reciprocal cross in that five hyperploid individuals with the recessive *bm* allele in all three chromosomes were found in the former while none were observed in the latter data. These five hyperploid *bm* plants arose from a crossover which transferred *bm* from a normal chromosome to the telocentric chromosome which later passed to the same pole as a *bm*-bearing normal chromosome 5. Similar gametes arising in microsporogenesis would fail to survive because of their relative inability to function in competition with haploid spores. Likewise 1.63 percent of the plants were primary trisomes homozygous for *bm* in progenies obtained when hyperploid plants were used as female parents while only 0.03 percent were found in similar progenies when the hyperploid plants were used as the male parents. There is no reason to believe that the actual frequency of such gametes is greatly different in the two sexes; it is simply a matter of the presence or absence of competition from haploid spores. The number of *bm* primary trisomes obtained when hyperploids were used as female parents is a measure of the frequency with which the two normal chromosomes 5 go to one pole while the telocentric chromosome passes to the other. Likewise the number of individuals hyperploid for the telocentric chromosome is a measure of the frequency with which one of the normal chromosomes passes to the same pole as the telocentric chromosome. The data show that there were 90 of the former to 1,671 of the latter type

of disjunction. This is precisely the type of behavior predicted from the cytological study of pairing and orientation on the spindle at metaphase I. As in the crosses where the hyperploid plants were used as the male parent, there occurred the unexpected class of secondary trisomes. Nine secondary trisomes were found; all of them were *Bm* which indicates that the telocentric chromosome was involved in the formation of the secondary chromosome.

The *bm* locus lies in the short arm of chromosome 5 close to the centromere. Distal to *bm* lies the *a2* allele some 10–12 crossover units removed. Data from plants carrying the dominant allele of the *a2* locus in the telocentric chromosome and recessive alleles in the two normal chromosomes 5 should parallel those reported for *bm* except that the greater crossover distance of *a2* from the centromere should alter the relative frequency of certain classes of offspring. These data are given in table 3. They agree very well with those for *bm*. The percentage of diploids with the dominant allele is 1.74 and 0.82 when hyperploids were used as the male and female parents, respectively. The *A2*-bearing normal chromosome arises through crossing over in the *A2*-centromere interval. The higher frequency of diploid *A2 a2* offspring obtained when hyperploids were used as the male parent in backcrosses suggests a higher crossover value in male than in female flowers. This is in agreement with unpublished data of the writer's which show for chromosome 5 significantly higher crossover values in male flowers as compared with the female. In both types of backcrosses the percentage of *A2 a2* individuals is several times greater than the percentage of *Bm bm* plants. This follows from the fact that *a2* is further removed than *bm* from the centromere.

In the data obtained when hyperploids were used as male parents, the percentage of hyperploid plants with the telocentric chromosome carrying *A2* is 0.42. In addition there were 2 (0.04 percent) individuals hyperploid for a telocentric chromosome with *a2*. These arose through crossing over followed by the functioning of a hyperploid grain and would consequently be found rarely. The total percentage of individuals hyperploid for the telocentric chromosome is 0.46 which is similar to the percentage of 0.51 in table 2 for the *bm* data. One primary trisome homozygous for *a2* was found. It could have arisen spontaneously in the diploid female parent or from the functioning of a pollen grain with two normal chromosomes 5 each with *a2*. As in the case of those hyperploid plants used in the *bm* experiments these also throw the unexpected class of secondary trisomes. There were 26 secondary trisomes in a total population of 5,450 or a percentage of 0.48 when hyperploid individuals were used as male parents. All 26 secondaries were *A2* in appearance which supports the conclusion

drawn from the *bm* data that the telocentric chromosome is involved in the formation of the secondary chromosome.

In the offspring of hyperploids used as the female parent 1,411 in a total of 5,605 individuals, a percentage of 25.17, were hyperploid for the telocentric chromosome. Thirteen hundred and eighty-six of them were phenotypically *A2* and 25 had *a2* in the telocentric as well as the normal chromosomes 5. Since the *A2* locus affects aleurone color an attempt was made to determine the genotypic constitution of all 1,386 plants hyperploid for the telocentric chromosome and carrying an *A2* allele by pollinating them with *a2* pollen. Successful pollinations were made on 1,159 plants. In all but one plant the aleurone ratios indicated the presence of a single *A2* allele borne by the telocentric chromosome. The single exception had *A2* in both the telocentric and a normal chromosome 5. This exceptional plant is a consequence of a crossover in the *A2*-centromere region between the telocentric and a normal chromosome, following which the two chromosomes involved in the crossing over passed to the same pole at anaphase I. One of the four possible combinations formed at the end of the second division would have an *A2* allele in both the telocentric and the normal chromosome 5. The 25 *a2* plants hyperploid for the telocentric chromosome also arose from crossing over in the *A2*-centromere region. If, following a crossover between the telocentric and one of the two normal chromosomes in this region, the assortment of the telocentric chromosome is at random with respect to the two chromosomes 5, there will result 3 $\frac{a}{a}$ to 1 $\frac{A}{A}$ combinations at the end of the second division. If the two crossover chromosomes always disjoin to opposite poles the expected ratio is 2:0 while a ratio of 1:1 is expected if they always pass to the same pole. The observed ratio of 25:1 indicates that the two crossover chromosomes usually pass to different poles. This correlation between crossing over and disjunction is similar to that found in triploid Drosophila. It should be mentioned that of the 1,385 individuals listed in table 3 as having their single *A2* allele in the telocentric chromosome only 1,158 were actually proven by genetic tests to be so constituted. However, since only one exceptional individual was found in the total of 1,159 tested, little error is introduced by this classification.

The percentage of *a2* plants hyperploid for the telocentric chromosome is 0.45 and the percentage of *bm* plants of similar chromosomal constitution, from table 2, is 0.09. This difference can be ascribed to the relative positions of the two loci with respect to the centromere. There were 48 primary trisomes homozygous for *a2*. This percentage of 0.86 is approximately

half the percentage of *bm* primary trisomes from a similar type of cross. The total frequency of individuals hyperploid for the telocentric chromosome in the *a2* data is 25.17, which is lower than the percentage of 30.35 found in the *bm* data. The reduced percentages of both primary trisomes and plants hyperploid for the telocentric chromosome in the *a2* data compared to the *bm* data suggest a lower frequency of pairing of the telocentric chromosome with the normal chromosomes 5 in the *a2* strain than in the *bm* strain. It is likely that this difference is genetically conditioned but nothing is known of its basis.

There were eight secondary trisomes in the progenies obtained using hyperploids as the female parent. All eight possessed the *A2* allele which again indicates that the telocentric chromosome was involved in the genesis of the secondary chromosome.

Data obtained from hyperploid plants possessing the dominant allele in the telocentric chromosome and the recessive allele in each of the normal chromosomes are the most illuminating since the dominant allele serves as a marker for the telocentric chromosome. However, in addition to these data a number of progenies were obtained from hyperploid plants which had a dominant allele in one of the two normal as well as in the telocentric chromosome. Data from a single combination of this type are presented in table 4. They will not be discussed in detail as they confirm in all respects the conclusions reached from the data in tables 2 and 3. The chief point of interest in these data is that secondary trisomes arise with a low but consistent frequency whenever the telocentric chromosome is present.

The data presented on the inheritance and behavior of the telocentric chromosome have been derived from individuals in which a single locus, either *a2* or *bm*, was followed in the telocentric chromosome. Data were obtained, in addition, from hyperploid plants in which both the *a2* and *bm* loci were marked by mutant alleles and the two long arms of the normal chromosomes were carrying the *Pr* and *pr* alleles.

The dominant allele at the *a2* locus produces aleurone color in the presence of *A*, *C* and *R* while *a2* results in colorless aleurone. In the hyperploid plants used these three complementary genes were homozygous dominant and only the *a2* locus was heterozygous, so a classification for *A2* and *a2* could be made before planting. The *Pr* and *pr* gene pair determines whether the color is to be purple or red, *Pr* conditioning purple and *pr* red color. In *a2* seeds it is impossible to classify for the *Pr pr* pair. The *a2* locus is also concerned in plant color so a check on the aleurone classification into colored and colorless was possible.

The constitution of the hyperploid plants for the three loci was *A2 Bm /A2 bm Pr/a2 bm pr;* the composition of the telocentric chromosome being listed first. These hyperploid plants were pollinated by triple recessive

pollen and the resultant seed divided into purple, red, and colorless classes. The ensuing progenies from the three classes of seed were classified for the *bm* character and for the various chromosomal types. These data are given in table 5. As a consequence of *Pr* lying in the same chromosome with *A2* while *pr* is in the *a2* bearing chromosome, the purple and red seed produced different percentages of the various classes. Among the progeny from *Pr* seed there were 471 diploid *bm* plants and only 199 from *pr* seed. The *A2 bm Pr* plants arose from non-crossover chromosomes (crossovers between the *A2 bm Pr* chromosome and the telocentric chromosome in the *A2 bm* region could not be detected) while the *A2 bm pr* individuals arose from crossovers between *pr* and *A2*. Equal numbers of plants hyperploid for the fragment should be found in the *Pr* and *pr* classes if the telocentric chromosome shows no preference with which normal chromosome 5 it disjoins in anaphase I. There were 281 and 350 hyperploid plants in the *Pr* and *pr* classes, respectively, which indicates no pronounced preferential assortment although the deviation of 35 from equality is somewhat suggestive. Another striking difference between the *Pr* and *pr* progenies is that there were 50 primary trisomes homozygous for *bm* among the *Pr* individuals while only four were found in the *pr* class. Those in the *Pr* class can be simply accounted for by non-disjunction of the two normal chromosomes. The four primaries homozygous for both *pr* and *bm* likewise arose from non-disjunction of the two normal chromosomes, but the fact that they came from gametes with two chromosomes 5 each with the recessive *pr* gene which was present in but one of the parental chromosomes indicates that a crossover in the *pr*-centromere interval took place between the chromatids of the two normal chromosomes followed by their non-disjunction in the first meiotic division. Since anaphase II is equational for the centromere, one-fourth of the combinations should carry two *pr* chromosomes while three-fourths should be *Pr Pr* and *Pr pr* in the ratio of 1:2 respectively. Approximately 12 of the 50 primary trisomes in the *Pr* class arose, therefore, in such a manner. There were two secondary trisomes present in both the *Pr* and *pr* classes. Of the 675 plants from *a2* seed all but two were diploids homozygous for *bm*. The two exceptions were hyperploid plants with a telocentric chromosome carrying *Bm*. These arose from a crossover in the *A2-Bm* region between the telocentric chromosome and the *a2-bm-pr* chromosome followed by their non-disjunction in anaphase I. One of the four possible combinations formed at anaphase 2 would possess a telocentric chromosome with the *a2 Bm* alleles and a normal chromosome of *a2 bm* constitution.

The genetic data presented in tables 2 to 5 inclusive are of interest in two respects. First, since it was possible to recognize all of the various chromosomal types arising as products of the meiotic process, extensive

genetic data were obtained which afforded a check on the observed cytological behavior of the telocentric and the two normal chromosomes in meiosis. Second, and of more interest since it is pertinent to the question of the stability of the terminal centromere, the data show that the telocentric chromosome was regularly involved in the genesis of a new chromosome equivalent to two short arms of chromosome 5 with a single, median centromere. Since adequate data have been presented on the frequency with which this new chromosome type arises, we will next consider the manner in which it originates.

ORIGIN OF SECONDARY CHROMOSOME

When plants hyperploid for the telocentric chromosome were used as the male parent a grand total of 19,242 offspring was obtained of which 86, or 0.45 percent, were secondary trisomes. Of a total of 17,175 offspring obtained when the hyperploid plants were used as the female parent there were 27, or 0.16 percent, secondary trisomes. This comparison is unfair since approximately 30 percent of the offspring in the latter crosses consist of hyperploids similar to the female parent, while less than one-half of one percent of such individuals were found when the hyperploids were used as the male parent. If the frequency of secondaries is calculated from the data obtained with the hyperploids as the female parent (omitting the hyperploid class from the total) the percentage of secondaries is 0.22. This value is about half that obtained when the hyperploid was the male parent. The relative frequencies of secondaries in the direct and reciprocal backcross data are in striking contrast to those of the other hyperploid types, namely the classes hyperploid for a telocentric chromosome or a whole chromosome 5. Although approximately 30 percent of the pollen grains possessed a supernumerary telocentric chromosome, only 0.46 percent of the offspring were hyperploid for this chromosome. It is certain that the hyperploid grains are at a great disadvantage against haploid pollen and only rarely succeed in functioning. Data have also been presented which show that grains hyperploid for a normal chromosome 5 are rarely functional in competition with haploid grains. It is likewise certain that there is little or no competition between euploid and aneuploid megaspores since the frequencies with which the different chromosomal types appear in the progenies obtained when the hyperploid is the female parent are reasonably close to those expected on the basis of pairing and disjunction at the first meiotic division in the microsporocytes. We are then faced with an anomalous situation in the frequency with which secondary trisomes appear when plants hyperploid for the telocentric chromosome are used as the pollen parent. There is no reason to believe that the frequency with which the secondary chromosome arises is enough

higher in the male flowers to account for the relatively high number of secondaries transmitted through the pollen, especially in view of the fact that pollen hyperploid for either the telocentric chromosome or chromosome 5 rarely functions even when present in large numbers. The possibility that two extra short arms of chromosome 5 have no detrimental effect on the pollen, while a single extra short arm is highly deleterious, and consequently that grains hyperploid for this secondary chromosome are as capable of functioning as haploid grains seems most unlikely. It is rendered untenable by the following experiment.

The secondary trisomes proved to be highly sterile in both the male and female flowers. Although the anthers have few aborted grains they are rarely extruded from the glumes and consequently shed no pollen. If, however, the mature anthers are removed and the pollen manually extracted, small quantities of viable grains can be obtained. When this pollen was applied to diploid silks a number of seeds were obtained. A total of 623 plants were grown from such seed and all proved to be diploids. It follows that those pollen grains hyperploid for the secondary chromosome were not able to function against haploid pollen. That they were present was proved by a study of the chromosomal complement of microspores at the first microspore division. Their frequency was not ascertained, however, because it was not always possible to differentiate between a supernumerary secondary chromosome and an extra normal chromosome 5. However, approximately 10 percent of the female progeny of a secondary trisome consist of secondary trisomes, so it is not unreasonable to assume that a like percentage, at least, of the pollen grains were hyperploid for the secondary chromosome. The failure to find a single secondary trisome in the offspring of a secondary used as the male parent makes it reasonably certain that the secondary chromosome so upsets the normal balance that the hyperploid grains are unable to successfully compete with haploid grains.

Before suggesting two possible mechanisms whereby the secondary chromosome may be transmitted through the pollen, it may be pertinent to consider the development of the male gametophyte which has been studied by a number of investigators. Essentially the story is as follows: The nucleus of the microspore divides to form a generative and a vegetative or tube nucleus. The generative nucleus divides again to form the two sperm cells. The mature male gametophyte or pollen grain at the time of anthesis contains three haploid nuclei—the vegetative nucleus, which is in a metabolic condition, and the two sperm nuclei. When the pollen grain germinates a pollen tube is extruded through the germ pore, enters the silk and grows down the silk towards the ovule. The vegetative nucleus assumes a position near the growing tip of the pollen tube and it is be-

lieved that the growth of the tube is under its control. It has been assumed that the two sperm are passive bodies playing no effective role in the activities of the pollen tube, merely being transported down the silk to the embryo sac.

The transmission of the secondary chromosome through the pollen may be readily accounted for if it is assumed that in a microspore with a telocentric chromosome the sequence of events is as follows: In the first microspore mitosis the telocentric chromosome splits equationally into two chromatids. Normally at anaphase each of these two chromatids possesses its own centromere and they pass to opposite poles. Rarely, however, the terminal centromere of the telocentric chromosome either fails to divide or divides transversely so that the two chromatids find themselves attached at their proximal ends to a common centromere. At anaphase this newly constituted chromosome with a median centromere and two identical arms passes to either the vegetative or generative pole. In the event that it moves to the generative pole, the end of the first microspore division finds a haploid vegetative nucleus and a generative nucleus hyperploid for the secondary chromosome. The two sperm formed by the division of the generative nucleus will each carry the secondary chromosome. A pollen grain of this constitution presumably would not be under any handicap during its stylar journey because it possesses a haploid vegetative nucleus. It carries, however, a sperm which will give rise to a secondary trisome if it fertilizes a haploid egg.

The postulated mis-division of the centromere of the telocentric chromosome has never been observed by the writer at anaphase of the first microspore division. DARLINGTON (1940), however, states that he observed the formation of iso-chromosomes in microspores of Fritillaria resulting from the delayed division of newly arisen telocentric chromosomes. An attempt was made to observe the phenomenon cytologically but the low frequency of its occurrence (about 9 in a 1,000 judging from the number of secondaries in the offspring) and the difficulty of identifying individual chromosomes at the microspore anaphase proved to be insuperable difficulties. While it was not possible to observe the genesis of the secondary chromosomes in the manner postulated the evidence at hand suggests that this mechanism or a similar one gives rise to the secondary chromosome.

In addition to the above hypothesis there is another possible way in which the telocentric chromosome might give rise to the secondary chromosome. KOLLER (1938), UPCOTT (1937) and especially DARLINGTON (1939) have shown that the centromere of an univalent chromosome sometimes divides transversely at meiosis in such a way that the two short arms are joined together and the two long arms are also attached to one piece of centromere. That is, the misdivision of the centromere gives rise to two

isochromosomes. In the case of the telocentric chromosome it has been observed that it is often unpaired by meiosis and that it sometimes fails to split equationally in anaphase I. It is possible, though it has not been cytologically demonstrated, and indeed it would be difficult to do so with certainty with a telocentric chromsome, that the centromere of an occasional telocentric chromosome divides transversely in either the first or second meiotic divisions to form an isochromosome with two identical arms. This newly formed isochromosome fails to reach either pole and forms a micronucleus. It must be further assumed that it persists until the microspore division where through its fortuitous position in the cell it is occasionally incorporated into the telophase group at the generative pole. The end result here is the same as in the first hypothesis, namely that the vegetative nucleus is haploid while the generative nucleus is hyperploid for the iso- or secondary chromosome. This hypothesis has the advantage that the misdivision of the centromere is postulated to occur in the meiotic divisions, where univalents of other plants have been observed to misdivide, and not in the somatic division of the microspore. While it has been necessary to invent the two hypotheses primarily to account for the transmission of the secondary chromosome through the pollen, it is highly probable that whatever mechanism is responsible for the origin of the secondary chromosome in the male flowers is also responsible for its origin in the female flowers.

The secondary trisomes originating from plants hyperploid for the telocentric chromosome have been studied cytologically. Figures C, D, E and F, Plate 3 and figures A, B and C, Plate 1 show that the secondary chromosome is composed of two short arms of chromosome 5 with a median centromere. A study of synapsis reveals that the order of loci in the secondary chromosome is *a b c d e* centromere *e d c b a* which is the order expected from the postulated mechanisms. When the secondary chromosome is a univalent but forms a chiasma between its two homologous arms a ring of one is found at diakinesis (figure C, Plate 3). The associations of the secondary and the two chromosomes 5 at pachytene shown in figures A, B and C, Plate 1 are explicable only if the 'secondary' chromosome consists of duplicate arms. The cytological behavior of these secondary trisomes is similar to that of the secondary for the short arm of chromosome 5 which arose spontaneously (RHOADES 1933a) in a stock disomic for chromosome 5. The genetic data disclose that the telocentric chromosome is involved in the formation of the secondary chromosome since with one exception (which can be accounted for by a crossover) the same alleles are present in the two chromosomes. (No secondary trisomes were found in the thousands of offspring from disomic sister plants.) These data also indicate that pollen hyperploid for one or two short arms of chromosome

5 rarely functions, yet the frequency of secondaries transmitted through the pollen is certainly no less than through the eggs. All of the above enumerated facts point to the correctness of the hypothesis that the secondary chromosome arises through mis-division of the centromere of the telocentric chromosome and as a consequence of this mis-division the generative and vegetative nuclei of the male gametophyte differ in their constitution.

Irrespective of the precise manner in which the secondary chromosome arises, it is a reasonable inference that its formation results from the instability of the terminal centromere of the telocentric chromosome. Whether its misbehavior consists of failure to divide or of a transverse instead of a longitudinal division cannot be stated with certainty but the observations of KOLLER (1938), UPCOTT (1937) and DARLINGTON (1939) on the transverse centromere division of univalent chromosomes at meiosis make the latter probability more likely. Evidence has been presented which suggests that in the formation of the secondary chromosome the mis-division of the terminal centromere occurs during or immediately following meiosis. An experiment was undertaken to determine if the telocentric chromosome was unstable in sporophytic mitoses. When plants hyperploid for the telocentric chromosome with the *Bm* allele, the two normal chromosomes 5 carrying *bm*, are used as female parents approximately 30 percent of the offspring are hyperploid for the telocentric chromosome. These individuals are *Bm* phenotypically because the telocentric chromosome bears the dominant allele. If, however, the telocentric chromosome or that part carrying the *Bm* locus is lost during the development of the sporophyte the recessive brown mid-rib character is expressed in the deficient portions of the plant. Three hundred hyperploid individuals were closely examined for the presence of *bm* sectors, and 22 or 7.3 percent were found possessing them. In these 22 plants the *Bm* allele present in the telocentric chromosome had been eliminated during development of the sporophyte. In several of the plants the deficient sectors extended into the tassel and a cytological examination was made of microsporocytes lacking the *Bm* allele. In one case the telocentric chromosome had been completely eliminated while in four other plants the telocentric chromosome had become diminished in size. In two of these instances there was a small fragment with a subterminal centromere; a second plant had a chromosome with a terminal centromere but only half the length of the parental telocentric chromosome; the third had an extremely small chromosome fragment consisting of nothing more than a terminal centromere with two or three chromomeres.

In the few cases studied cytologically no indication was found that the *bm* variegation was due to a reciprocal translocation occurring in a somatic

cell and resulting in somatic segregation such as JONES (1938) reports for the endosperm of maize.

A similar experiment was conducted in which the telocentric chromosome was marked with the dominant *A2* allele while the normal chromosomes 5 carried the recessive allele. The *B* and *Pl* alleles were also present in this stock so the *A2 B Pl* plants had a purple plant color. If *A2* was lost during the development of the sporophyte the tissue deficient for this allele would be brown instead of purple. Five hundred and one purple hyperploid plants were examined at maturity for brown sectors and 31 or 6.2 percent possessed them. The *a2* sectors must have arisen through the loss of *A2* in telocentric chromosome. The size of both *bm* and *a2* sectors varied from small to large. No cytological study has as yet been made of the *A2* losses. It appears, however, from the study of certain of the *Bm* losses that an unchanged telocentric chromosome was present in the early embryo and that some alteration occurred during development because the non-deficient cells possessed a complete telocentric chromosome while the deficient cells had a reduced or missing telocentric chromosome. If elimination of the telocentric chromosome in somatic tissue sometimes occurs through the transverse division of the centromere in a manner similar to that believed to happen at meiosis, the sectorial plants should show an asymmetry produced by the marked differences in appearance and texture between diploid and secondary tissues. In no variegated plant was tissue characteristic of the secondary found. Secondary chromosomes may arise in somatic cells but no evidence that they do has been obtained. The simple explanation of the transverse division of the centromere will not account for the origin of the diminutive chromosomes. It must be admitted that the nature of these structural changes is unknown but that they are a consequence of the terminal position of the centromere can be argued with some reasonableness. The secondary chromosome is a direct product of the telocentric chromosome but has a median rather than a terminal centromere. If the instability of the telocentric chromosome is due to some factor other than the unusual position of its centromere it would be expected that the secondary chromosome would also be unstable. However, nearly 200 secondary trisomes have been obtained during the course of these studies and no evidence of instability of the secondary chromosome, exhibited either as asymmetrical sectors of growth or variegation, has been found. It is the writer's experience, and he understands also of other maize students, that sectors due to loss or somatic segregation are rarely found in the sporophyte. This is true of trisomic as well as disomic plants. Apparently a chromosome with an interstitial centromere is more stable than one with a terminal centromere.

THE STRUCTURE OF THE CENTROMERE

The centromere appears at pachytene in maize chromosomes stained with aceto-carmine as a simple body with a homogeneous, translucent appearance. There is no suggestion of the compound nature which SCHRADER found for the centromeric region of Amphiuma. In Amphiuma the centromere is a compound body composed of the commissural cup and spindle spherule. The spindle spherule is connected with the half spindle component. Presumably this is a function reserved for the spherule and in case of its loss the commissural region would be unable to form a half spindle fiber. It would seem that the centromeric region of a maize chromosome lacks this specialization of its component parts because MCCLINTOCK (1932, 1938) found that both parts of a fractured centromere were able to function in a normal manner. NEBEL (1939) believes that the centromere is a compound body consisting of three parts: a central achromatic body, the chromatic kinetic bodies (equivalent to spindle spherules), and the chromatic connecting chromomeres of the chromonemata with the achromatic body. If the centromere is broken he assumes that the kinetic body will be regenerated by that part of the achromatic body not retaining it after breakage. In the case of the maize contromere it is difficult to determine the formation or loss of an invisible body. It seems probable that in maize there is no differentiation of the centromeric region into recognizable structures having specialized duties but that on the other hand any part of the centromere region, providing it is not attached to an inordinately large piece of chromatin, is able to function normally. In this connection it should be noted that MCCLINTOCK found that each part of a fractured nucleolar-organizer body was able to function.

DARLINGTON (1939) from a consideration of the transverse division of the centromere of unpaired chromosomes reached certain conclusions concerning its internal structure. He concluded that it possesses a dual nature consisting of a fluid and a fibrous element. The fibrous elements or centrogenes normally control the plane of division or 'explosion' of the fluid element. Since the fibrous elements lie across the centromere the fluid will usually divide in the plane of division of the centrogenes. DARLINGTON accounts for the observed misdivision of the centromeres of univalent chromosomes by assuming that the centrogenes apparently divide after the chromonemata and misdivision is due to their failing (exceptionally) to divide in time for the explosion of the centric fluid which is precocious in univalents at meiosis. DARLINGTON'S conclusions regarding the internal structure of the centromere are admittedly speculative, but it seems to the writer that he is justified in assuming some internal organization within the centromere to account for its normally orderly longitudinal division. Whether or not his conception of the cause of misdivision of the centro-

mere is correct there is no doubt that misdivision does occur since it has been observed cytologically by DARLINGTON, KOLLER, and UPCOTT in addition to the evidence presented in this paper on the genesis of the secondary chromosome.

In considering the ways by which the secondary chromosome could have arisen from the telocentric chromosome, it was suggested that a terminal centromere might be 'sticky' and would occasionally become attached to another terminal centromere especially if they were in close proximity for a considerable time. That is, if two telocentric chromosomes were in the same cell their centromeres might fuse to form a metacentric chromosome. Following RANDOLPH'S (1932) technique, root tips of seedlings hyperploid for the telocentric chromosome were submerged in hot water to induce doubling of the chromosomes. After treatment the root tips were fixed and sectioned. A dozen clear polar views at metaphase of cells with the double number of chromosomes were found and in each the two telocentric chromosomes although lying parallel to one another were clearly separate. Though the heat treatment produced a restitution nucleus with double the number of chromosomes, the terminal centromeres of the two telocentric chromosomes did not fuse after lying in juxtaposition for some hours. These observations are of such a fragmentary nature as to merit little weight but they indicate that the formation of the secondary chromosome occurs when the centromere of the telocentric chromosome has misdivided and not from 'unsaturation' of terminal centromeres.

LEVAN (1938) believes that the division of the centromere is delayed by the alkaloid colchicine. When root tips were treated with colchicine he found at metaphase what he describes as c-pairs formed by the attachment of the two daughter chromosomes to their undivided centromere. The inactivation of the spindle apparatus produced by colchicine is believed to be connected with a delay in the division of the centromere. (This is in agreement with SCHRADER'S and DARLINGTON'S conception of the centromere as playing an important rôle in the development of the spindle.) After a time the centromere divides and the two daughter chromosomes come to lie free from each other but in parallel alignment. Since DARLINGTON believes that the misdivision of the centromere of unpaired chromosomes at meiosis is due to the failure of the centrogenes to divide in time for the explosion of the centric fluid, it seemed possible that a delayed division of the centromere of the telocentric chromosome produced by colchicine treatment might invariably result in the formation of the secondary chromosome. Healthy root tips of plants hyperploid for the telocentric chromosome were submerged in an 0.2 percent aqueous solution of colchicine for one hour. Twenty-four hours later the material was fixed and sectioned. A number of clear figures were found in which doubling had oc-

curred but in no case were the two telocentric chromosomes attached at the centromeric region. More extended observations of both colchicine and heat treated material might have shown an occasional secondary chromosome but there is reason to doubt if either treatment would be effective. Misdivision of the centromere of univalent chromosomes occurs either at or immediately after the meiotic divisions. The centromere of a univalent is, however, at this time in a pecularíar situation compared to the paired centromeres of a bivalent and its misdivision results from an aberrant precocious attempt to divide one mitosis in advance of the usual time. In the colchicine and heat-treated material all of the chromosomes are subject to the same forces concomitantly and there is no more delay in the division of the centromere of the telocentric chromosome than of the other centromeres of the chrosome complement.

ON THE ORIGIN OF SECONDARY TRISOMES

Secondary trisomes were first reported by BELLING and BLAKESLEE (1924) in Datura. Since each chromosome is two-armed and the secondary chromosome consists of two homologous arms incorporated into a single chromosome there are two possible secondary trisomes for each chromosome. In Datura all 12 of the possible primary types have been found but only 14 of the 24 secondaries have been discovered (BLAKESLEE and AVERY 1938). In a number of other plants including maize and *Nicotiana sylvestris* all or nearly all of the primary types have been isolated. Secondaries have been rarely reported. The writer (1933a) described a secondary for chromosome 5 in maize and one has been reported by PHILP and HUSKINS (1931) in Matthiola. Recently, GOODSPEED and AVERY (1939) believed they had found several secondary trisomes in *Nicotiana sylvestris* but their classification was based on the appearance of the plants and not on cytological examination so final judgment must be withheld concerning the true nature of their supposed secondaries.

BELLING and BLAKESLEE (1924) suggested that the secondaries originated from a reversed synapsis of two homologous chromosomes and that a crossover occurred at the only place where homologous parts were together which would be the centromere. This hypothesis can be rejected as improbable. BLAKESLEE and AVERY (1938) suggest "that unequal crossing over between parallel sister strands in such a way as to retain spindle attachment points for each newly organized chromosome which has been formed by joining together by their broken ends the two similar halves of the strands affected" might account for the origin of the secondaries. However, the results reported here and by the writer in 1938 as well as the cytological observations by KOLLER, UPCOTT and DARLINGTON make it probable that the secondaries arise through transverse division of the

centromere. While only in the maize secondary has it been established that the secondary chromosome is a strict isochromosome it seems probable that the others are also of a similar structure and have all arisen through misdivision of the centromere. Secondaries might arise directly from an unpaired chromosome by misdivision of its centromere as has been observed cytologically by the investigators mentioned above, or from misdivision of a telocentric chromosome as reported in this paper. The low frequency with which secondary trisomes arise from the telocentric chromosome makes it likely that in many instances the secondaries arise directly from the misdivision of unpaired atelocentric chromosomes although a telocentric chromosome is a potential source of secondaries. If, in organisms with no telocentric chromosomes in the normal complement, secondaries come only from telocentric chromosomes, their frequency would be the product of the probability of a telocentric fragment arising and the probability that once having arisen it would be transformed into a secondary chromosome. There are no data available from which the correlation of the frequency with which telocentric chromosomes arise through misdivision or other causes and the frequency of secondary trisomes can be determined. Since both telocentric chromosomes and isochromosomes were observed by KOLLER, UPCOTT and DARLINGTON as products of the misdivision of the centromere, it is not likely that all secondaries come progressively from telocentric chromosomes followed by misdivision of the centromere. Judging from the data reported in this paper for the maize telocentric chromosome the misdivision of its centromere is a relatively rare event. However, in considering the origin of secondary or isochromosomes it is of interest to note that recently DARLINGTON (1940) followed the behavior of telocentric chromosomes arising through misdivision of the centromere at meiosis in the first microspore division. He states that "Following misdivision of the centromere at meiosis in diploid and triploid Fritillaria new telocentric chromosomes are formed whose broken ends rejoin within the centromere. This type of chromosome is delayed at metaphase and anaphase in the pollen grain mitosis. It may then either break again at the centromere or pass without separation to the pole as a new isochromosome." It is not known whether or not this delayed division of the Fritillaria telocentrics in the pollen grain division also exists in the sporophytic divisions of the following generation. In the case of the maize telocentric reported in this paper there is good reason to believe that the type of misdivision occurring in the gametophyte division does not happen in the somatic division of the sporophyte.

The data compiled by BLAKESLEE and AVERY (1938) on the frequency of secondaries from diploids and related primaries are in agreement with DARLINGTON'S thesis that misdivision of the centromere of univalent chro-

mosomes gives rise to secondaries or isochromosomes. In a trisomic plant one of the three homologous chromosomes is often unpaired which is precisely the condition favoring misdivision. Actually, the Datura workers found that the secondaries were thrown by related primaries 14 times as frequently as by diploids.

The secondary chromosome formed by the misdivision of the telocentric chromosome has two identical arms which is true of the attached X's in *Drosophila melanogaster*. It is doubtful, however, if the attached X's arose by a misdivision of the centromere, although such an origin is a possibility, since L. V. MORGAN (1938) has shown that two X chromosomes may become attached by replacement through crossing over of the two arms of a Y chromosome by X's.

HÅKANSSON (1932) reported a chromosome in Triticum with like ends. While he believes it arose through crossing over in a duplicated segment and its two arms therefore not wholly equivalent, it is possible that it is a true isochromosome and arose through misdivision of the centromere. LOVE (1939) reported ring univalents in Triticum which may be isochromosomes. HUSKINS and SPIER (1934) and LOVE (1938) have reported a chromosome in Triticum with a terminal centromere due to the loss of one arm. It is of some interest to know if these telocentric chromosomes will give rise to isochromosomes. BLAKESLEE and AVERY (1938) state that a telocentric chromosome in Datura consists of the ·11 part of the 11·12 chromosome. If this chromosome has a truly terminal centromere it should form an occasional 11·11 secondary.

RANDOLPH (1928a) has described a type of supernumerary chromosome in maize known as the B-type. MCCLINTOCK (1933) found that the centromeres of the B-types were terminal although DARLINGTON (1937) believes them to be sub-terminal. DARLINGTON'S conclusions were drawn from a study of somatic metaphase plates where he observed a constriction near one end which he interpreted to be the centric constriction. MCCLINTOCK, however, studied the pachytene stage where a much clearer picture of the morphology is obtainable and her published photograph of two paired B-types shows a terminal centromere. While it is possible that there are different kinds of B-types and that those which DARLINGTON studied possessed sub-terminal centromeres, it is not unreasonable to suppose that the constriction observed by DARLINGTON marks the junction of the euchromatin and the deeply staining pycnotic bodies (heterochromatin?) so clearly seen in the pachytene chromosome. While there is some dispute concerning the location of the centromere of the B-type chromosome, if we accept MCCLINTOCK'S findings, as the writer does, it is of some interest that RANDOLPH (1928b and unpublished) has found a series of diminutive chromosomes. All of them probably descended by fragmentation from an

original B-type since LONGLEY (1938) found that a diminutive chromosome frequently synapsed with B-types. This behavior of the B-type corresponds to the fragmentation of the telocentric chromosome reported in this paper and it is not improbable that in both instances the unstability is due to the terminal location of the centromere.

The data reported in this paper on the behavior of the telocentric chromosome leave no doubt that this chromosome is unstable. It gives rise to an isochromosome through misdivision of its centromere and it was also found to undergo structural changes in somatic divisions leading to loss or diminution in size. The mechanism of the latter changes is unknown but the greater frequency of their occurrence in the telocentric chromosome makes it probable that they are a result of the terminal position of the centromere.

S. NAWASCHIN in 1916 declared that no chromosome in the normal complement of any organism possessed a terminal centromere. This is true for plants and may hold for animals. If the behavior of all terminal centromeres is similar to the one reported in this paper the absence of telocentric chromosomes is understandable because the unstability of terminal centromeres would lead to the elimination of chromosomes possessing them.

ACKNOWLEDGMENT

During the course of the four years in which the data reported here were obtained it was necessary to determine cytologically the chromosome constitution of literally hundreds of plants in order to insure that the visual classification of the various chromosome types were accurate. Without the efficient help of VIRGINIA H. RHOADES this would have been too arduous a task to have been accomplished and the writer wishes to express his appreciation of her invaluable assistance.

SUMMARY

Maize plants hyperploid for a telocentric chromosome consisting of the short arm of chromosome 5 produce an occasional secondary trisome. The supernumerary chromosome of the secondary trisomes consists of two short arms of chromosome 5 attached to a median centromere. It was shown through the use of mutant genes lying in the telocentric chromosome that it was involved in the formation of the secondary chromosome. The frequency with which secondary trisomes were found when plants hyperploid for the telocentric chromosome were used as the pollen parents was 0.46 percent, while their frequency was only 0.22 percent when the same plants were used as female parents. Pollen hyperploid for either one or two short arms of chromosome 5 rarely functions successfully in competition with haploid grains. It is suggested, therefore, that the secondary chromo-

some arises at meiosis from a transverse division of the centromere of the telocentric chromosome, and that it is occasionally incorporated into the generative nucleus during the first microspore division. The vegetative nucleus would be haploid and pollen tube growth normal but the two sperm would transmit the secondary chromosome.

Data have been obtained which indicate that the telocentric chromosome undergoes structural changes in somatic cells. The production of secondary or isochromosomes at meiosis from the telocentric chromosome and its loss and modification in somatic tissue show that a terminal centromere is unstable. Such a telocentric chromosome would tend to be eliminated by natural selection. This instability may apply to all telocentric chromosomes and account for the fact that telocentric chromosomes are rarely, if ever, found in the normal chromosome complement of any organism.

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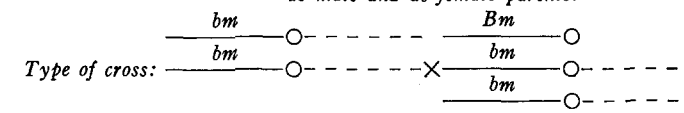
TABLE 2

Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross:  *and reciprocal*

CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
Bm / *bm* / *bm*	18	0.25	10	0.18
bm / *bm* / *bm*	7166	98.91	3738	67.68
Bm / *bm* / *bm*	37	0.51	1671	30.26
bm / *bm* / *bm*	0	0.00	5	0.09
Bm / *bm* / *bm*	0	0.00	0	0.00
bm / *bm* / *bm*	2	0.03	90	1.63
Bm *Bm* / *bm* / *bm*	21	0.29	9	0.16
bm *bm* / *bm* / *bm*	1	0.01	0	0.00
	7245	100.00	5523	100.00

TABLE 3

Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross: a2/a2 ○- - - - - - × A2/a2 ○- - - - - - and a2/a2 ○- - - - - - *and reciprocal*

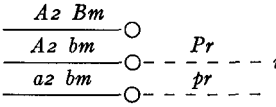
CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
A/a ○- - - - - -	95	1.74	46	0.82
a/a ○- - - - - -	5303	97.30	4092	73.00
A/a ○ ; a/a ○- - - - - -	23	0.42	1385	24.71
A/A ○ ; a/a ○- - - - - -	0	0.00	1	0.02
a/a ○ ; a/a ○- - - - - -	2	0.04	25	0.45
A/a ○- - - - - - ; a/a ○- - - - - -	0	0.00	0	0.00
a/a ○- - - - - - ; a/a ○- - - - - -	1	0.02	48	0.86
A A/a ○ ; a/a ○- - - - - -	26	0.48	8	0.14
a a/a ○ ; a/a ○- - - - - -	0	0.00	0	0.00
	5450	100.00	5605	100.00

TABLE 4

Summary of data when $\frac{Bm}{Bm}$ ○ / $\frac{Bm}{bm}$ ○ - - - - - - *individuals were used as the male in backcrosses to diploid bm plants. The phenotypes of the different chromosomal classes are in the second column.*

CHROMOSOMAL CONSTITUTION	PHENOTYPE	OFFSPRING OBTAINED	
		NUMBER	PERCENT
———○- - - - - - / ———○- - - - - -	*Bm*	2596	48.06
———○- - - - - - / ———○- - - - - -	*bm*	2755	51.00
———○ / ———○- - - - - - / ———○- - - - - -	*Bm*	22	0.40
———○ / ———○- - - - - - / ———○- - - - - -	*bm*	0	0.00
———○- - - - - - / ———○- - - - - - / ———○- - - - - -	*Bm*	0	0.00
———○- - - - - - / ———○- - - - - - / ———○- - - - - -	*bm*	0	0.00
———○——— / ———○- - - - - - / ———○- - - - - -	*Bm*	29	0.54
———○——— / ———○- - - - - - / ———○- - - - - -	*bm*	0	0.00
		5402	100.000

TABLE 5

Summary of data when  *individuals were used as the female in backcrosses to diploid a2 bm pr plants.*

CHROMOSOMAL CONSTITUTION	PHENOTYPE	NUMBER OF OFFSPRING
	a bm	673
	a Bm	0
——————○- - - - - -	*A Bm Pr*	2
——————○- - - - - -	*A Bm pr*	1
	A bm Pr	471
	A bm pr	199
	a bm	0
	a Bm	2
——————○	*A Bm Pr*	278
——————○- - - - - -	*A Bm pr*	350
——————○- - - - - -	*A bm Pr*	3
	A bm pr	0
	a bm	0
	a Bm	0
——————○- - - - - -	*A Bm Pr*	0
——————○- - - - - -	*A Bm pr*	0
——————○- - - - - -	*A bm Pr*	50
	A bm pr	4
	a bm	0
	a Bm	0
——————○——————	*A Bm Pr*	2
——————○- - - - - -	*A Bm pr*	2
——————○- - - - - -	*A bm Pr*	0
	A bm pr	0

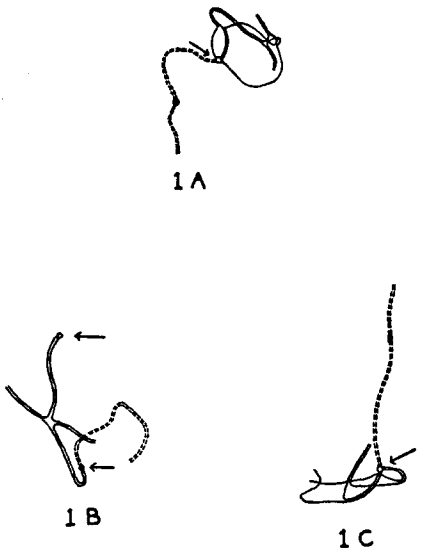
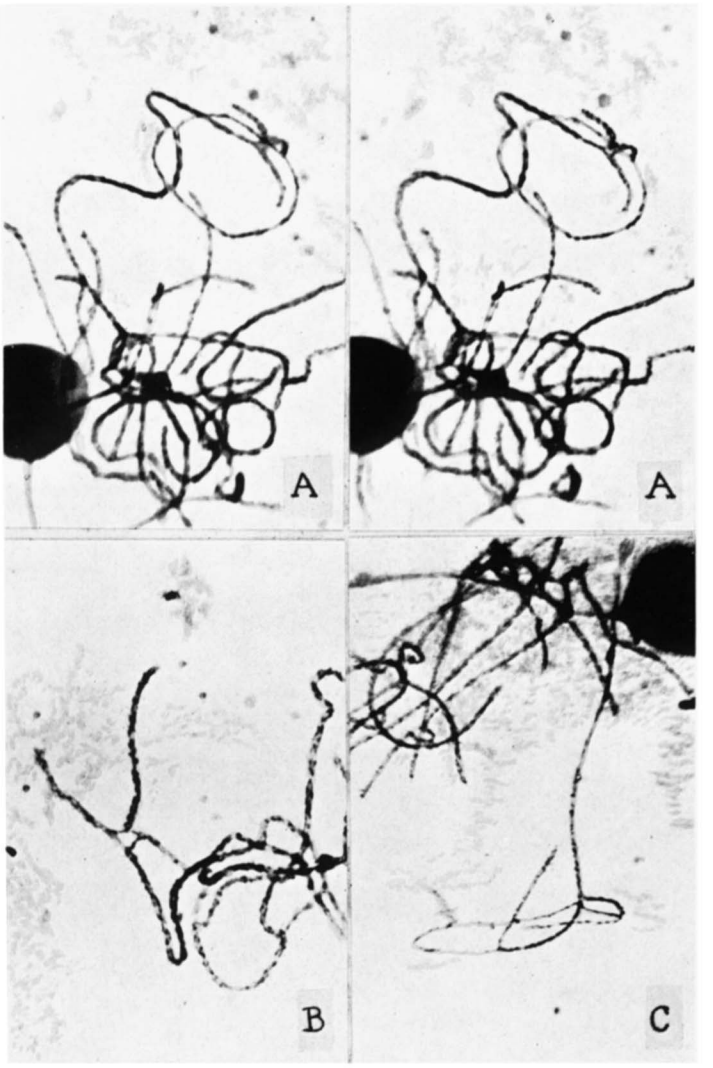
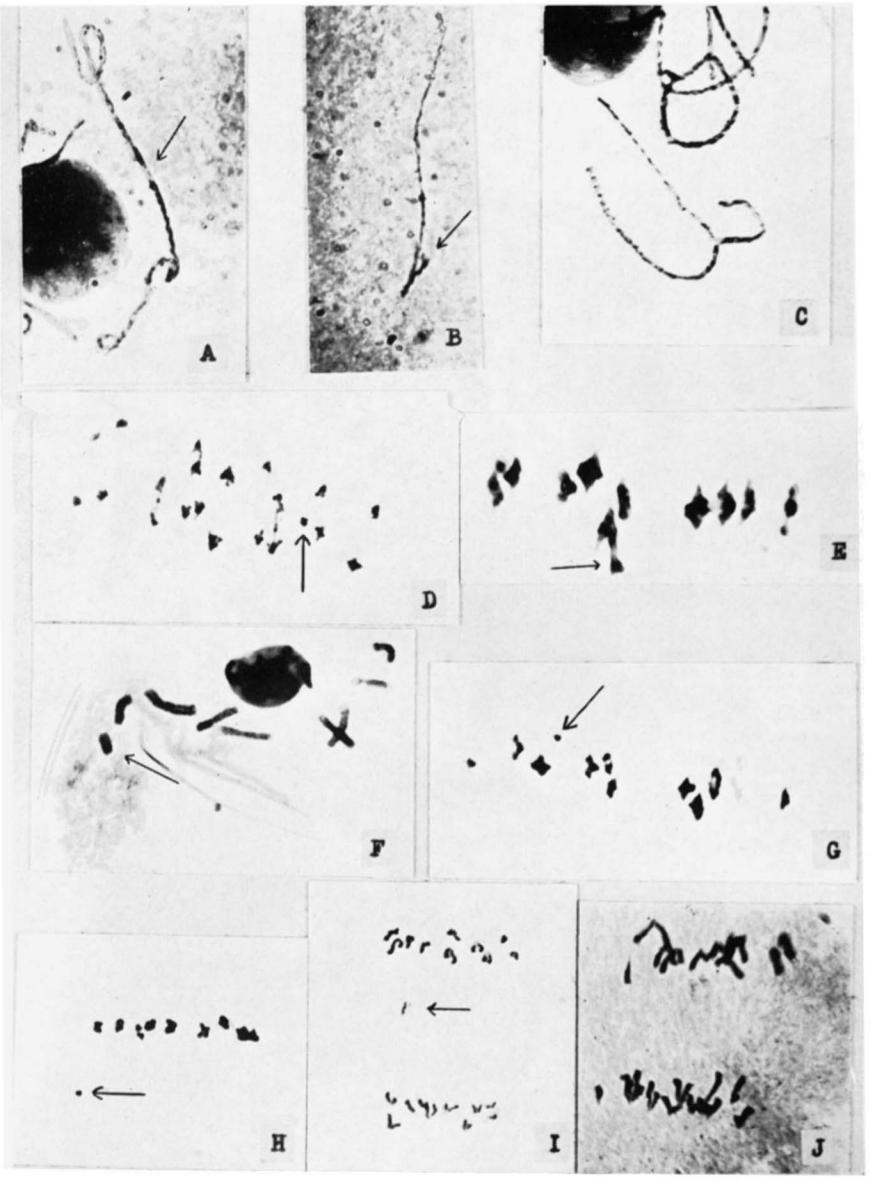


FIGURE 2.—Diagrammatic sketches of the pachytene configurations shown in Plate 1. The centromeres are represented by bulges and are indicated by arrows. The long arms of chromosome 5 are shown by broken lines while the short arms are indicated by solid lines. Figure 1 A corresponds to figure A, Plate 1, figure 1 B to figure B, and figure 1 C to figure C in Plate 1.

EXPLANATION OF PLATE 1

Figures A, B and C show synapsis at pachytene between the secondary chromosomes and the two normal chromosomes 5. The two photomicrographs of figure A are at different levels. Figure B is from a secondary trisome that arose in a stock disomic for chromosome 5 (RHOADES 1933a). The more intimate pairing seen in figure B is not a characteristic difference between this secondary trisome and those arising from the telocentric chromosome. See figure 2 for interpretation.


A
A
B
C


A
B
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D
E
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G
H
I
J

EXPLANATION OF PLATE 2

FIGURE A.—Photomicrograph of two chromosomes 5 paired at pachytene. The centromere is indicated by arrow.

FIGURE B.—Photomicrograph at pachytene of unpaired telocentric chromosome. The equational split is evident at the distal end. The terminal centromere is indicated by the arrow and was clearly terminal.

FIGURE C.—Photomicrograph at pachytene of telocentric chromosome with its centromere stuck to the centromeres of two paired chromosomes 10. The distal end of the telocentric has a foldback, that is, it is paired hon-homologously in this figure. There is no suggestion that the telocentric chromosome is two-armed.

FIGURE D.—Early anaphase showing disjunction of the paired homologues while the unpaired telocentric chromosome is still on the plate. In late anaphase it may separate equationally and its two chromatids (daughter univalents) migrate to different poles.

FIGURE E.—Metaphase I showing trivalent composed of telocentric and two chromosomes 5. The orientation of the telocentric chromosome, indicated by arrow, is such that it will disjoin with a normal chromosome 5.

FIGURE F.—Late prophase in microspore hyperploid for the telocentric chromosome which is indicated by arrow.

FIGURE G.—Metaphase I showing 10 bivalents and unpaired telocentric chromosome which has congressed on the spindle.

FIGURE H.—Metaphase II with 10 dyads on the equatorial plate and a daughter univalent, arising from the equational separation of the telocentric chromosome in anaphase I, lying off the plate.

FIGURE I.—Anaphase II with 10 monads passing to each pole while a daughter univalent lags. Origin of daughter univalent same as in figure H.

FIGURE J.—Anaphase II with 11 monads passing to each pole. In the preceding anaphase the telocentric chromosome was a member of a trivalent group and underwent a reductional division. The two daughter univalents of the telocentric chromosome are rod-shaped because of their terminal centromeres while the other monads are V- or J-shaped.

EXPLANATION OF PLATE 3

FIGURE A.—Metaphase I showing that the centromere is divided at its poleward tip. Each chromatid will form its own half-spindle component but the bulk of the centromere does not appear to be divided.

FIGURE B.—Anaphase I showing centromeres divided at poleward tips. As in figure A the bulk of the centromere does not appear divided.

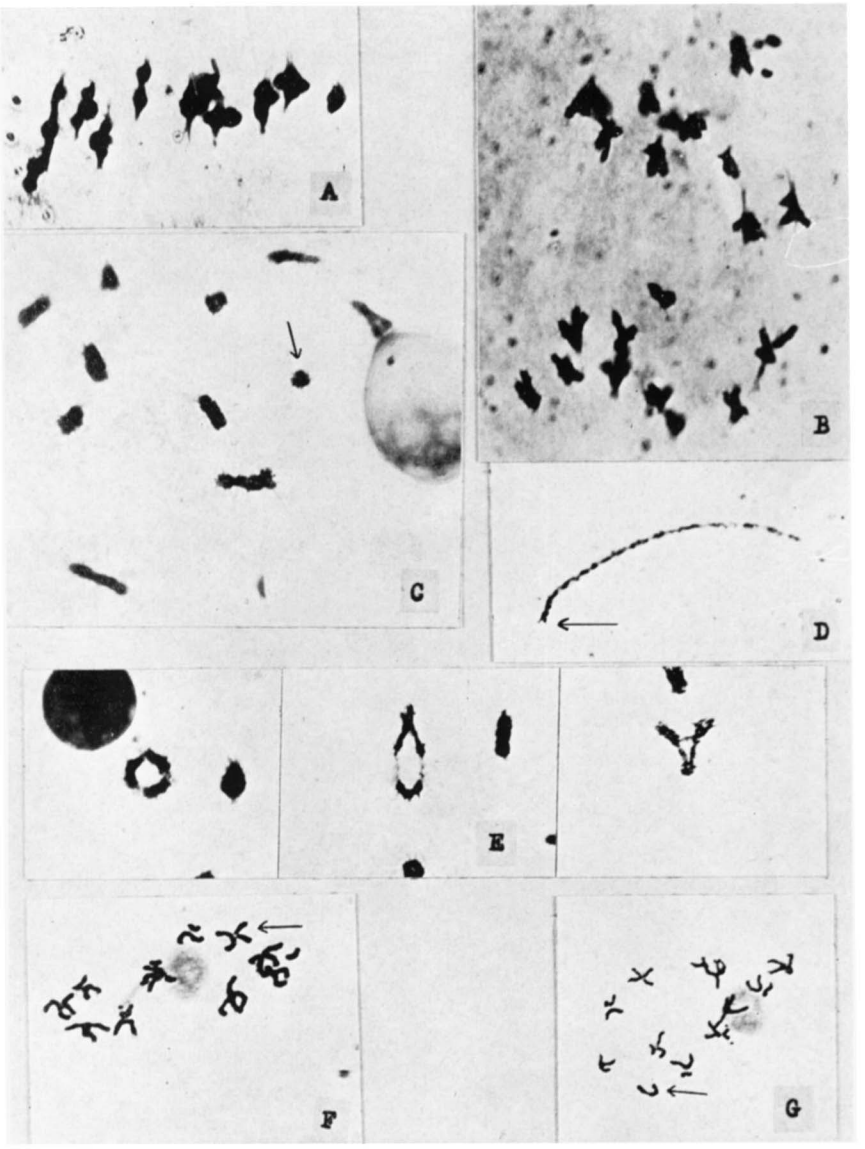
FIGURE C.—Diakinesis in secondary trisome with the secondary chromosome present as a ring of 1. This configuration results from chiasma formation between its two homologous arms.

FIGURE D.—Pachytene stage showing pairing of the two identical arms of secondary chromosome. The median centromere (see arrow) has a terminal position because of the synapsis of the two homologous arms. This configuration will give a ring of 1 at diakinesis.

FIGURE E.—Diakinesis in secondary trisome. Three ring configurations produced by pairing of secondary chromosome with the two normal chromosomes 5. These rings of 3 are produced by pachytene associations shown in Plate 1. In the leftmost ring terminalization is complete while it is only partially so in the middle figure and in the rightmost figure there has been little if any movement of the chiasmata.

FIGURE F.—Prophase II with the secondary chromosome indicated by arrow. Since the two arms of this chromosome are alike the dyad appears as an X-shaped element with all four arms of the X of equal length. In the dyad to the left of the secondary the two short arms appear continuous as do the two long arms. The unstained centromere lies between the two chromatids at the center of the X. The rod-shaped body to the right is a B-type daughter univalent.

FIGURE G.—Prophase II showing the two chromatids of a telocentric chromosome. Since the two chromatids appear continuous the centromere must be forced to one side at this stage. If the centromere was not strictly terminal an X-shaped figure would be produced at prophase II as is the case for the rest of the chromosome complement.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