THE STRUCTURE AND MEIOTIC BEHAVIOR OF THE DIFFERENTIATED CHROMOSOMES OF TOMATO

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FOR a number of years the elongate chromosomes of salivary gland nuclei of Diptera and the pachytene chromosomes of plants have proved of use in researches in cytogenetics. The pachytene and later meiotic chromosomes of the tomato offer interesting material for cytological and perhaps cytogenetic studies because of their morphology. The chromosomes of the tomato are strikingly differentiated into regions differing in diameter and staining capacity. In addition, the differential regions seem to show differential behavior during meiosis with respect to pairing, location of chiasmata, and contraction.

The arms of the tomato chromosomes are divided into two sorts of regions. One sort is adjacent to the centromere, is relatively broader, and stains very deeply with iron aceto-carmine. Because of its staining capacity, this sort of region will be called a chromatic zone. The other sort forms the distal portion of the chromosome arms, is relatively narrower, and stains very lightly. This sort of region will be called an achromatic zone. The chromatic zones have a very distinct pattern of specific chromomeres while the achromatic regions possess very delicate chromomeres which are difficult to observe. The transitions from the chromatic to the achromatic zones are very abrupt. The achromatic regions all terminate in small, but very distinct knobs, or telochromomeres which thus form the ends of the chromosomes.

The chromosomes of the tomato seem to correspond most closely in structure to the meiotic chromosomes of Agapanthus (BELLING 1928; DARLINGTON 1933; GEITLER 1933), Oenothera (JAPHA 1939; MARQUARDT 1937), Pellia (JACHIMSKY 1935), and Sphaerocarpus (LORBEER 1934). Each of the features mentioned above for the tomato has been reported for one or more of these plants. It is possible to see all of these characteristics clearly in the tomato.

Because of its importance as an economic plant, the tomato has been the subject of numerous cytological investigations. Although achromatic zones have been recognized at pachytene (LESLEY and LESLEY 1935) and achromatic threads at diakinesis pictured by many workers, all previous investigators have concerned themselves largely with the chromatic material. Technical difficulties have apparently prevented previous workers from obtaining a clear picture of chromosome structure in the tomato. Some of these difficulties have recently been overcome by the development of a mordanting modification of the aceto-carmine smear technique by DR. MARTA S. WALTERS. Even with this method which gives an unusually intense stain to the chromatic zones, the

achromatic zones are occasionally so poorly stained that they are difficult to observe.

In the following report the structure of the chromosomes at pachytene will be described first. After this, the behavior during the meiotic sequence, with respect to pairing, formation of chiasmata, and contraction, will be described.

MATERIALS AND METHODS

All plants used in this study were from a highly inbred line of the English greenhouse forcing variety, Sutton's Best. Some of the plants originated from X-rayed pollen, the 700-series, and the others were untreated, the 800-series. The irradiation was performed to produce a translocation which was necessary for part of the study of chromosome contraction, and this translocation was studied in the single F_1 plant, 725-1. Studies of the nucleolus-organizing chromosome were made with three plants, 808-18, 725-1, and 724-1; the 700-series plants were used inasmuch as good preparations were available, and the paired homologues at pachytene showed no detectable aberrations. Measurements of the nucleolus-organizing chromosome were made of at least several chromosomes at pachytene in all plants from which later stages were taken. Studies of chromosome pairing at zygotene and of chiasmata frequencies at diakinesis were made only on untreated material in order not to confuse the normal behavior with that which might be caused by aberrations.

Petals and sepals were removed from buds before they were fixed for twenty-four hours in three parts absolute alcohol to one part glacial acetic acid. The writer wishes to express his appreciation to DR. MARTA S. WALTERS for information on the subsequent technique. This method was adapted to the tomato by her, and has given excellent results in this and other laboratories. After fixation the buds were soaked in two or three changes of tap water for thirty minutes to an hour. They were then mordanted for thirty minutes to an hour in four percent iron alum after which they were again soaked in several changes of tap water for at least an hour. After this mordanting the buds would not keep well and were used at once. Contents of an anther were squeezed out into a small drop of iron aceto-carmine, the debris removed, and the pollen-mother cells broken up by stirring with a needle. A cover-slip was placed on top of the drop, and the slide was heated over an alcohol burner until it was hot to the touch but not boiling. The slide was then placed between several layers of paper toweling and pressed vigorously, with care not to move the cover-slip. Slides can be sealed with a convenient preparation or made permanent in euparal (DARLINGTON and LACOUR 1942). All photomicrographs in this report were made from permanent preparations.

Photomicrographs were made with a Bausch and Lomb K camera which gave an enlargement, with a 90× objective and 15× ocular, of 1200×. Enlargements to 2000× or greater were made with an Omega DII enlarger. All negatives were Eastman Contrast Process Ortho, developed in D-11. Most of the papers used in printing were F4 and F5.

PACHYTENE STRUCTURE

The structure of the tomato chromosome is most clearly evident at the pachytene stage of meiosis. The chromosomes typically show the following structural characteristics: (1) a centromere, (2) chromatic regions on each side of the centromere and of varying extent for each arm, (3) achromatic distal regions, also of varying length, and (4) small terminal knobs, or telochromomeres, which appear to terminate all chromosome arms. These characteristics will be considered in detail.

The chromatic and achromatic zones appear to possess some of the properties of hetero- and euchromatin, respectively. The behavior of the chromatic material in the resting nucleus is complex. Consequently the simple descriptive terms will be used in this report, and some of the similarities of the regions of the tomato chromosomes to hetero- and euchromatin as they have been described in other organisms will be referred to in the discussion.

The centromere resembles that of maize in being an elongate and achromatic structure (McCLINTOCK 1931). In some cells all twelve centromeres may be seen, but will show more clearly in certain chromosomes than in others (figs. 2, 6, chromosomes 6, 7, 8, 9, 10, 11, 12).

The chromatic zones appear to consist of relatively large chromomeres which stain deeply. Between these large chromomeres, which vary somewhat in size along the length of the chromosome, narrower achromatic zones are sometimes apparent. The two chromosomes most readily identifiable at pachytene are the nucleolus-organizing chromosome and a chromosome with a very short and characteristically patterned chromatic zone. Both will be used to illustrate the structure of the chromosomes. The nucleolus-organizing chromosome has a short arm which is completely chromatic and a long arm consisting of a chromatic zone and a very long terminal achromatic region. The long arm begins with a very heavy chromomere adjacent to the centromere; this heavy chromomere is followed by a less heavily chromatic zone which is followed again by large chromomeres (figs. 3, 7, 9, 22). The chromatic zone of the chromosome with the short chromatic region consists of only three chromatic elements, a very large chromatic structure which begins the short arm and two heavy chromomeres in the proximal part of the long arm (figs. 2, 6, 8, 21).

The achromatic zones of the tomato chromosomes constitute the distal portions of all the chromosome arms except the short arm of the nucleolus-organizing chromosome. They are very weakly stained threads which in many cases are difficult to differentiate from the cytoplasm in which they are usually spread out on smearing (figs. 8, 21). The boundary between the chromatic and achromatic zones is usually sharply defined. The chromomeres of the narrow transition regions between the chromatic and achromatic zones are stained deeply but are distinctly smaller than those of the chromatic zones (figs. 2, 6, upper arms of chromosomes 1 and 12). The achromatic regions possess chromomeres which are very difficult to detect because of their light stainability (figs. 42–49). No chromosome has been observed which is not achromatic for at least half its length. The chromosome with the short chromatic region obviously has

a much greater achromatic than chromatic length (figs. 2, 6, 8, 21). The same is true of the nucleolus-organizing chromosome (table 1, figs. 3, 7, 9, 22). Other chromosomes may possess obviously shorter achromatic regions (figs. 3, 7).

The chromosomes typically end with a small chromatic knob, or telochromomere. The telochromomeres are smaller than the chromomeres of the chromatic zones and must be in sharp focus to be seen. For example, the telochromomere at the end of the short arm of the chromosome with the short chromatic region is clearly visible in one photomicrograph (figs. 2, 6), but not in the other (figs. 8, 21). The demonstration that all twenty-four chromosome arms do end in telochromomeres will require the identification of all twelve chromosomes. However, over seventeen ends with telochromomeres have been counted in single nuclei and the five lowermost achromatic zones in figs. 2 and 6 may all be seen to terminate in telochromomeres. In addition, no chromosome end which has been seen clearly has been found not to possess a telochromomere. Thus, if not a universal feature of the chromosome ends of the tomato, the telochromomere is at least very typical.

The region of the nucleolus-organizing chromosome associated with the nucleolus is in the short arm. This arm is apparently the only one of the complement which consists entirely of chromatic material. In mitotic divisions (figs. 30–34) the distal end of this arm is clearly dissociated from the rest of the chromosome to form a small satellite. In meiosis, on the other hand, the distal end comes to lie adjacent to the remainder of the short arm, and no separate satellite is formed. The region connected with the nucleolus can be identified because the rest of the chromosome is frequently pushed away from the nucleolus on smearing. A shallow and short constriction may sometimes be seen at this region, and beyond this constriction the distal end of the short arm frequently appears as a lump or bump. How much of this distal end is concerned with the formation of the nucleolus is not known at present. The only point which can be determined with certainty is the region closest to the centromere to which the nucleolus is attached. This is the region marked by the shallow constriction. The distal end of the chromosome which occurs beyond the point where the nucleolus is attached to the chromosome may be seen in figs. 6 and 7, and is diagrammed as the solid region in figs. 2 (chromosome 3) and 3. The length of the distal segment is 1.9 microns at pachytene, or about 40 percent of the length of the short arm (table 1). In mitotic prophase and metaphase, the satellite formed about 50 percent and 42 percent of the lengths of the short arms, respectively. LESLEY and LESLEY (1935) and LESLEY (1938) have been concerned in particular with the satellite region of the chromosome, and have discovered varieties of tomato differing in satellite size. The satellite of Sutton's Best seems definitely to belong to the "short" rather than to any of the "long" classes.

ZYGOTENE PAIRING

In cells of late zygotene or early pachytene in which pairing is only partially completed, the chromosome regions seem to be differentially paired according to their chromaticity. Such cells are not abundant, so the stage in which the

pairing differential may be observed clearly is probably of short duration. However, there are usually several chromosome arms showing this behavior in one nucleus. Both the centromere and the distal achromatic zones pair while the chromatic zones usually remain completely or partially unpaired (figs. 1, 5).

TABLE 1

Lengths in microns of regions of the nucleolus-organizing chromosome during meiotic and mitotic stages (see also diagrams, fig. 51)

STAGES	REGION						
	SATELLITE*	TOTAL SHORT ARM	CENTROMERE	CHROMATIC OF LONG ARM	TOTAL CHROMATIC**	ACHROMATIC OF LONG ARM	TOTAL CHROMOSOME
Pachytene	1.9	4.8	1.6	4.6	11.0	26.9	37.9
S.E.***	*(10)*	*0.21*	*0.15*	*0.15*	*0.36*	*1.31*	*1.33*
Early Diakinesis	—	—	—	—	3.8	6.2	10.0
S.E.	—	—	—	—	*0.24*	*0.32*	*0.33*
Middle Diakinesis	—	—	—	—	2.3	4.2	6.5
S.E.	—	—	—	—	*0.10*	*0.26*	*0.30*
Late Diakinesis	—	—	—	—	1.9	2.9	4.8
S.E.	—	—	—	—	*0.06*	*0.12*	*0.13*
Interphase	—	1.2	0.5	1.2	2.9	0.0	2.9
S.E.	—	*0.08*	*0.07*	*0.08*	*0.15*	—	*0.15*
Mitotic prophase	0.9	1.7	0.0	0.9	2.6	1.6	4.2
(n)***	*(4)*	*(2)*	*(4)*	*(2)*	*(4)*	*(2)*	*(2)*
Mitotic metaphase	0.6	1.4	0.0	0.9	2.3	0.0	2.3
(n)	*(2)*	*(2)*	*(2)*	*(2)*	*(2)*	*(2)*	*(2)*

* The "satellite" measurements for pachytene are the lengths from the apparent region of attachment of the nucleolus to the distal end of the short arm.

** The lengths of the centromere are included in all total chromatic lengths because the centromere can not be distinguished at later meiotic stages.

*** The standard error is given in italics below each of the averages; n = 15 for pachytene, and n = 25 for later meiotic stages. Where standard error is not given, the number of measurements made, n, is given in parentheses.

Later stages, of course, show an apparently tight synaptic union along the entire length of the chromosome.

The observed interruptions in pairing, however, may be thought to be the result of the usual sequence of chromosome pairing in the tomato without respect to any possible differential behavior of the chromatic regions. In order to determine whether or not chromatic differentiation plays a part in chromosome pairing, the results obtained from observations on pairing were compared statistically with the results expected on the basis of several hypotheses of chromosome pairing. These hypotheses assume that pairing is independent of chromaticity.

Cells were studied in order from a slide with late zygotene material on it. The first 40 chromosome arms having unpaired segments were measured with

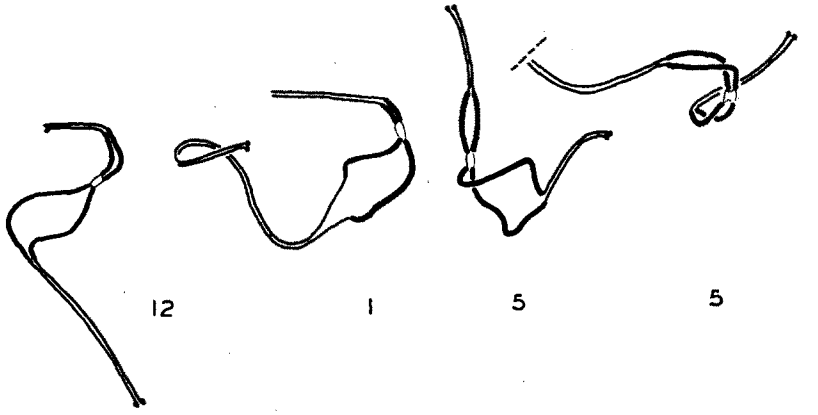

FIGURE 1.—Diagram of fig. 5. Late zygotene or early pachytene. Four bivalents, at 12, 1, and two at 5 o'clock in the photograph. Part or all of the chromatic material of each bivalent is not yet paired while achromatic regions and centromeres are paired.

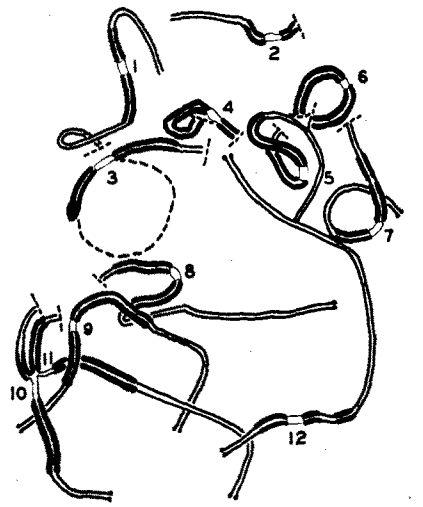

FIGURE 2.—Diagram of fig. 6. Pachytene. All twelve chromatic regions are apparent. Chromosomes are numbered for reference purposes only, and the numbers do not imply identification of all of the chromosomes. Note the chromosome with the short chromatic region, No. 12; it may be followed its entire length. No. 3 is the nucleolus-organizing chromosome; only its chromatic material is visible. Nos. 10 and 11 show centromeres lying close together.

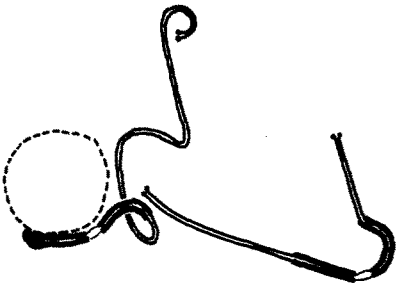

FIGURE 3.—Diagram of fig. 7. Pachytene. The nucleolus-organizing chromosome and one other may be followed their entire lengths.

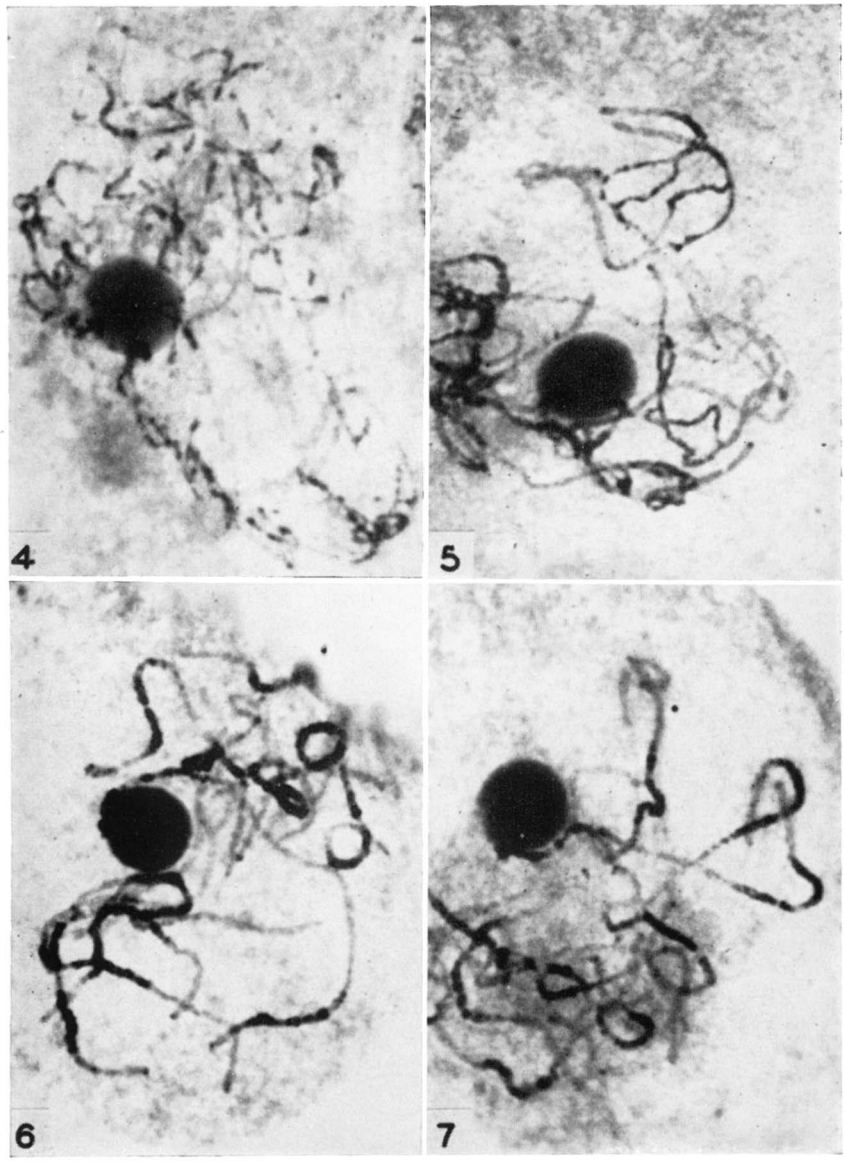

PLATE I

Zygotene to pachytene. 2000×. Fig. 4. Zygotene. Fig. 5. Unpaired chromatic zones at early pachytene. See diagrams, fig. 1. Fig. 6. The chromosome with the short chromatic region at pachytene, chromosome No. 12, see diagram fig. 2. All twelve chromatic regions are visible. Fig. 7. Nucleolus-organizing chromosome and another bivalent at pachytene. See diagram, fig. 3.

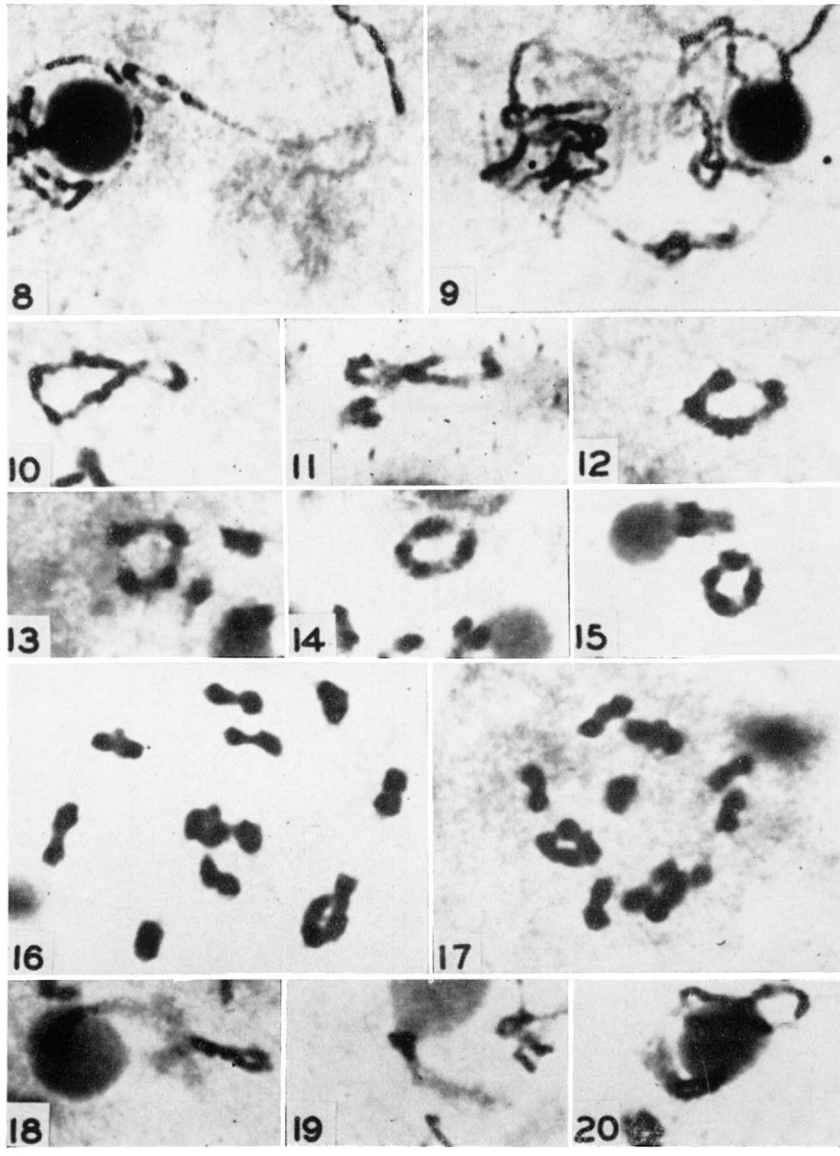

PLATE II

Pachytene to prometaphase. 2000×. Fig. 8. The chromosome with the short chromatic region at pachytene. See diagram, fig. 21. Fig. 9. Nucleolus-organizing chromosome at pachytene. See diagram, fig. 22. Figs. 10–11. Translocation ring-of-four chromosomes at early diakinesis. Figs. 12–14. Translocation at middle diakinesis. Fig. 15. Translocation at late diakinesis. Figs. 16–17. Translocation at prometaphase with rest of complement, at five o'clock in fig. 16 and at eight o'clock in fig. 17. Figs. 18–20. Nucleolus-organizing chromosome at early diakinesis.

an ocular micrometer. For each of these arms, the lengths of the chromatic and achromatic zones, and the length and position of the unpaired segment were recorded. The results of these measurements are illustrated graphically in fig. 23. In the construction of this graph, each chromosome arm was given a standard length divided equally into chromatic and achromatic zones regardless of the actual lengths or proportions of these regions. The horizontal lines indicate

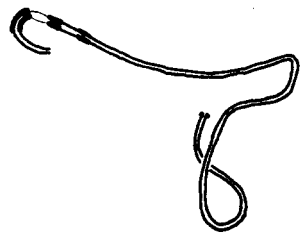

FIGURE 21.—Diagram of fig. 8. Pachytene. The chromosome with the short chromatic region. Note that in the loop, the achromatic material is difficult to differentiate from the cytoplasm.

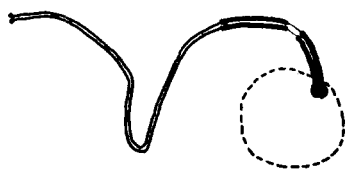

FIGURE 22.—Diagram of fig. 9. Pachytene. The complete nucleolus-organizing chromosome.

the relative portions of the chromatic and achromatic regions which formed the unpaired segments.

Thirty-seven of the forty arms showed unpaired segments at least in part in the chromatic zones. In 30 of the 40 arms, the distal end of the unpaired segment was at or near the transition region of the chromatic and achromatic zones. In these 30 arms, pairing ends near the transition region within ten percent of the chromatic zone or ten percent of the achromatic zone. In the further discussion these regions will be referred to as the ten percent transition regions; their limits are indicated by the vertical dotted lines in fig. 23, and the 30 arms make up the group enclosed by the bracket.

It is now necessary to determine whether the occurrence of 30 distal ends of unpaired segments at or near the transition zones is or is not in reasonable agreement with the postulate that the tendency of a region to pair is independent of its chromaticity. Expected frequencies of this occurrence were therefore computed on the basis of the different assumptions described below. All the assumptions lead to expected frequencies less than those observed.

According to the first assumption, pairing takes place at random along the length of the chromosome. In this event, the probability that the distal end of an unpaired segment in any chromosome arm will be observed to be in a given region is directly proportional to the length of this region. In addition, the

assumption is made that the positions of pairing of different chromosome arms observed in the same cell are uncorrelated. According to this assumption the distal end of only one out of ten unpaired segments would be observed within the ten percent transition regions. However, unpaired segments entirely within the achromatic zones are more difficult to detect than those at least partly within the chromatic zones. Even if lack of pairing was never detected in distal regions of the achromatic zones, however, not more than one out of five of the non-paired segments observed should have its distal end at or near the transition region.

According to the second assumption, chromosome pairing begins simultaneously at the ends of the chromosome arms and moves toward the centromere regardless of the pairing behavior of the centromere itself. As pairing progresses the unpaired segments would decrease in length until, at different times for different chromosome arms, the distal ends of these segments would come to lie in the ten percent transition regions. At a particular stage at which the cells were fixed, there would be a maximum number of these ends lying within the ten percent transition regions. The expected frequencies, to be discussed below, were computed on the extreme assumption that all the cells were fixed at exactly this most favorable stage.

According to the second assumption, part *a*, pairing may proceed on a relative basis. When one chromosome arm is paired for half its length, other chromosome arms are also paired for about half their lengths regardless of dissimilarities in actual length. From the measurements of the lengths of the same 40 chromosome arms on which pairing observations were made, it was possible to determine that the largest number of transition regions, 17, occurred within the ten percent interval of 57 to 67 percent of the length of the arm from the end to the centromere. Therefore the maximum number of distal ends of unpaired segments occurring at or near the transition region, under the extreme assumption mentioned, would be expected to be 17 out of 40. If some or all of the cells were fixed at a somewhat different stage, the expectation would be lower.

According to the second assumption, part *b*, pairing starts simultaneously in all arms and proceeds at equal rates on the basis of actual length. When one chromosome arm is paired for two microns from the end, other chromosome arms are paired for approximately two microns regardless of whether two microns is a small or large part of the chromosome arm. Ten percent of the average length of all the arms, 1.4 microns, was chosen as the test interval. It was then possible to determine that the largest number of transition regions, 14, occurred within the ten percent interval of 7.2 to 8.6 microns from the end toward the centromere. The number of distal ends observed to be lying within 0.7 micron to either side of the transition region was 27.

On the basis of the first assumption, the maximum expectation of eight distal ends within and 32 without the ten percent transition region can be compared with the observations of 30 and ten, respectively. A chi square test indicates that the deviations are far greater than can reasonably be attributed to chance (table 2).

On the basis of assumptions 2a and 2b the expected values are 17 and 23, and 14 and 26, respectively. The corresponding observations are, in the same order, 30 and 10, and 27 and 13. For various reasons, a valid chi square test can not be made to compare these observed with expected values. To do so would require that each observation was independent. A chi square test would be permissible (1) were each chromosome arm observed in a different cell, (2) if the expected values were derived from the entire chromosome set, and (3) if each chromosome arm had an equal probability of being selected from a given cell. In this case, however, deviations from these requirements would tend either to decrease or not affect chi square. The observation of more than one chromosome arm in a given cell would tend to decrease chi square since if a

TABLE 2

Tests of hypotheses of chromosome pairing which assume independence of chromaticity. For further explanation, see text

DISTAL END OF UNPAIRED SEGMENT	HYPOTHESIS 1		HYPOTHESIS 2a		HYPOTHESIS 2b	
	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.
In ten percent transition zone	10	32	10	23	13	26
Elsewhere in chromosome arm	30	8	30	17	27	14
Chi square (Yates' corr. used)	72.22		15.98		17.17	
Chi	8.50		3.99		4.14	
P<	1×10^{-9}		0.000, 1		0.000, 1	

first observation showed a distal end within a ten percent transition region, the second would have a decreased probability of showing the same thing, and so forth. Secondly, since the expected frequencies are based on the actual chromosomes examined rather than on a complete set of chromosomes, the actual chi square is likely to be small. Finally, under the present circumstances, an accumulation of the same or similar arms in successive cells would increase the expected frequencies at the same rate as the observed. Thus the chi squares computed to test hypotheses 2a and 2b are to be regarded as minimum values, and the probabilities as maximum values rather than valid estimates of these values (table 2). The very low values of the two probabilities, both less than 0.0001, indicate that assumptions 2a and 2b, together with the extreme assumption that all cells were fixed at the same most favorable stage, are not tenable.

Because of the marked deviations of the observed values from those expected on the basis of all the hypotheses given above, it seems unlikely that an hypothesis which does not include the differential behavior of the chromatic and achromatic regions will satisfactorily explain chromosome pairing in the tomato.

In addition to pairing before the adjacent chromatic zones, the centromeres show one other type of differential behavior. Centromere regions of non-homologous chromosomes were frequently found to be adhered or lying on top of each other at pachytene, but not at later stages. Such behavior was noted in both X-rayed and untreated material (figs. 2, 6, centromeres 10, 11). In addition, this behavior made necessary a postponement of a study of the translocation at pachytene because the translocation seemed to occur near the centromere regions of both chromosomes, and to bring the non-homologous centromeres close enough together to induce a very frequent adherence. There was insufficient material available to differentiate the translocation configurations from those caused by adhering centromeres. In Agapanthus, DARLINGTON (1933) observed similar non-homologous centromere fusions which he believed resulted from an interlocking during pairing. Such interlocking seems not to be true in tomato because no interlocking configurations were seen to occur at later stages, and in many cases the centromeres were merely lying close together.

CHIASMATA AT DIAKINESIS AND METAPHASE; THE CHROMOSOMES AT METAPHASE AND ANAPHASE

In the tomato, chiasmata may be observed readily at late diakinesis. In order to determine their approximate number and position, all of the chiasmata in ten cells at late diakinesis were recorded. This count gave a total of 162 chiasmata of which 49 were interstitial and 113 terminal. This material therefore had an average of 1.35 chiasmata per bivalent, and a terminalization coefficient of about 70 percent. The nucleolus-organizing chromosomes of these cells, when considered alone, had nine interstitial and four terminal chiasmata, or an average of 1.3 chiasmata per bivalent and a terminalization coefficient of only 30 percent.

A single cell in which all of the chiasmata may be seen is pictured in fig. 41. In this cell there was a total of 18 chiasmata, 13 of which were terminal (T) and 5 interstitial (I). In this cell, as in the counts given in the preceding paragraph, the number of chiasmata classified as interstitial was a minimum. For example, the bivalent at eleven o'clock in fig. 41 may have had an interstitial instead of a terminal chiasma at its left end.

In the cells examined, all of the chiasmata observed were in the achromatic regions. In order to test the hypothesis that chiasmata are more abundant in the achromatic than in the chromatic regions, another series of observations was made on the short arm of the nucleolus-organizing chromosome which consists entirely of chromatic material. This region of the chromosome is difficult to work with because of the attachment to the nucleolus and because of the occasional presence during most of diakinesis of fine chromatic strands which connect the two short arms. These fine chromatic strands usually appear to be subterminal, and therefore they cannot be confused with chiasmata when they are in this position. At late diakinesis, the association of the chromosome with the nucleolus has considerably weakened, and most of the chro-

matic strands have disappeared. Those strands which do remain are difficult to distinguish from chiasmata because the contraction of the chromosome makes them appear to be terminal.

The length of the short arm of the nucleolus-organizing chromosome at pachytene is 4.8 microns while that of the long arm is 31.5 microns. If the number of chiasmata formed is proportional to chromosome length, then the short arm of the nucleolus-organizing chromosome would be expected to have $(4.8/31.5)\times1.3$ or an average of 0.2 chiasmata per bivalent. Even if the observed chromatic strands are counted as chiasmata, the number of chiasmata in the short arm of the nucleolus-organizing chromosome falls significantly

TABLE 3

Test of significance of difference between number of chiasmata (or chiasma-like chromatic strands) in the short arm of the nucleolus-organizing chromosome at late diakinesis and the number expected on the basis of pachytene length

	OBS.	EXP.
Chromosomes separate	54	44
Chromosomes superimposed	(3)	—
Chiasma or chromatic strand	1	11
Totals	55 (58)	55
Chi square (Yates' corr. used)	10.25	
P<	0.01	

short of this expectation (table 3). Although it may be disputed whether or not chiasmata are formed at all in the chromatic zones, it seems very unlikely that they are to be found there with the same frequency, on a length for length basis, in which they are found in the achromatic regions.

The interstitial chiasmata persist until metaphase (figs. 36–39). During the early anaphase movements of separation of the bivalents, part of the chromosomes are stretched out between the separating members. As the chromosomes move still further apart, the material which previously appeared to be considerably extended between them disappears suddenly as though it had been released from tension (figs. 38, 39). At middle anaphase, the chromosomes appear more uniform than at previous stages of meiosis, and can only occasionally be differentiated into lighter and darker staining regions (fig. 40).

CHROMOSOME CONTRACTION; MITOTIC CHROMOSOMES

In the tomato, the achromatic regions seem to constitute only a small portion of chromosome length at late prophase of either mitosis or meiosis. The achromatic regions at these stages occupy a much smaller proportion of the

total length of the chromosomes than they do at earlier meiotic stages. The relatively greater shortening of the achromatic zones may be accounted for in several ways. The contraction of the achromatic zones may be proportionately greater than that of the chromatic zones. By the term contraction is meant simply a shortening or reduction in length of a chromosome or section of a chromosome. This term is not meant to imply any mechanism, such as spiralization, which might be responsible for the reduction. On the other hand, the regions of the achromatic zones near the chromatic material may change during prophase, become deeply stainable, and thus become indistinguishable in appearance from the chromatic material. If this were true, the residual achromatic material would obviously occupy a relatively smaller proportion of the length of the late prophase chromosome than would the chromatic material and the altered achromatic material together. Measurements of a chromosome with visible marker points were used to determine which of these alternatives was occurring during prophase.

The expected results from each of these two types of change is illustrated diagrammatically in fig. 50. If the achromatic material were to become reduced in length to practically nothing while the chromatic material were only moderately reduced in length, the asymmetric chromosome pictured above would eventually change to a symmetric chromosome (arrow A). If the achromatic material were to become indistinguishable from the chromatic material, while both it and the chromatic material were contracting, the resultant chromosome should be markedly asymmetric (arrow B).

The nucleolus-organizing chromosome is composed of two arms, a short, chromatic arm, and a long arm containing a short, proximal, chromatic region and a long achromatic zone. The two chromatic regions on either side of the centromere are of about equal length, while the distal achromatic zone is much longer than the two chromatic zones added together (figs. 3, 7, 9, 22; table 1). In addition, the part of the chromosome connected with the nucleolus marks off a distal knob or bulge occupying more than one-third of the end of the short arm. The limits of this distal region could not be accurately determined in all of the chromosomes which were selected for measurement, but sufficient measurements were made to give a fairly good estimate of its size (table 1). As previously mentioned, this region of the chromosome does not form a satellite at meiosis, but forms a very distinct satellite during mitosis.

During diakinesis, the chromatic region forms one end of the nucleolus-organizing chromosome, but can no longer be marked by either the satellite or the centromere (figs. 18–20, 24–27). At interphase, however, and at mitotic prophase and metaphase, the centromere region is clearly apparent (figs. 28, 29, 32, 34). If the achromatic material has really contracted to an extremely small length, the nucleolus-organizing chromosome should have arms of approximately equal length at interphase and at mitotic metaphase. In this case the chromatic material alone would form a symmetric chromosome. If the achromatic material had become altered to resemble the chromatic material, the final product should be an asymmetric chromosome with the arm bearing the nucleolus much shorter than the other.

Measurements of nucleolus-organizing chromosomes at interphase indicate that the two arms are of approximately equal length (table 1). At mitotic metaphase, the arm bearing the satellite is somewhat longer than the opposite arm. The satellite does not form a thread of the same thickness as the rest of the chromosome, and it seems for this reason that the nucleolus-bearing arm is longer at these stages. The achromatic material can be seen during mitotic divisions for other chromosomes as well as the nucleolus-organizing chromosome (fig. 35). The changes in length of the various regions of the nucleolus-organizing chromosomes may be easily summarized in a chart (fig. 51). The change from a marked asymmetry at pachytene to a nearly symmetric chromosome at interphase and mitotic metaphase indicates that the long achro-

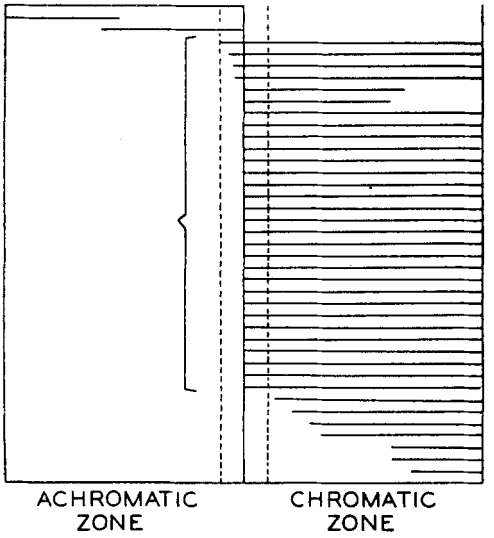

FIGURE 23.—Chart showing extent of unpaired segments in 40 chromosome arms at late zygotene. All chromosomes have been given a standard length divided into equal chromatic and achromatic zones. The horizontal lines indicate the proportionate extent and location of the unpaired segments. The vertical dotted lines indicate the ten percent of the chromatic zone and the ten percent of the achromatic zone adjacent to the transition between the two zones (continuous vertical line at center). The bracket includes the 30 arms which had distal ends of unpaired segments occurring within the ten percent limits.

matic zone has contributed practically nothing to the length of these chromosomes. Therefore, the achromatic material apparently has not acquired, either from its own production or from the chromatic zone, more than a very small amount of stainable material.

By following both the nucleolus-organizing chromosome and the translocation, the relative rate of change of length of the chromatic as compared with the achromatic zone may be determined. When lengths of chromatic regions are plotted against achromatic regions, an approximately straight line relationship is apparent, and a straight line may be easily fitted to the points by means of least squares (fig. 52). The much wider spacing of the stages for the trans-

location than for the nucleolus-organizing chromosome follows from the fact that the translocation measurements include four chromosome lengths (two bivalents) while those of the nucleolus-organizing chromosome, only one (one chromosome from each bivalent) (figs. 10–17). The chromosomes in the translocation seem to be smaller than the nucleolus-organizing chromosome.

The slopes of the two lines in fig. 52 are remarkably similar. In both cases the chromatic zones decrease in length at a much slower rate than do the achromatic zones. In both cases, the slopes of the lines do not go through the origin, indicating that there would be sizable pieces of chromatic material left even if the achromatic zones were to contract to zero. These residual chromatic lengths would be equal to 1.03 and 6.09 microns for the nucleolus-organizing chromosome and the translocation, respectively.

It is next of interest to consider the relative rates of chromosome contraction on the basis of the almost complete disappearance of the achromatic material as described above. If the achromatic material was converted to material indistinguishable from chromatic material, the chromosome as a whole would still be present after the achromatic material had apparently disappeared. If a straight line relationship were produced as a result of such a conversion, the straight line would not be expected to pass through the origin. If both chromatic and achromatic zones shortened at different rates but with no change of achromatic to chromatic material, the straight line would be expected to pass through the origin. The seemingly contradictory facts that the observed straight line does not go through the origin and that the achromatic material is not converted to chromatic material lead to a postulate of a very simple mechanism to account for them. This postulate will not only account for the observed relations but can be cytologically verified in part. The writer wishes to express his sincere thanks to DR. EVERETT R. DEMPSTER for suggesting this mechanism.

If the chromatic zones consist of alternate regions of heavy chromomeres resistant to contraction, and achromatic interchromomeric regions capable of contracting, then the chromatic zones would contract only until further contraction was blocked by contact of the chromomeres with one another (fig. 53). If the achromatic interchromomeric bands contracted at the same rate as the material of the achromatic zones, the relative rates of contraction of the chromatic and achromatic zones would give a straight line relationship. The slope of this straight line would be dependent upon the lengths of the chromatic and achromatic zones and the amount of interchromomeric material in the chromatic zones. Because of the blocking of the contraction of the chromatic zone by the heavy chromomeres, this straight line would not pass through the origin. That the chromatic zones do consist of chromomeres separated by rather lengthy achromatic interchromomeres may be seen clearly at zygotene in the tomato (fig. 4). Presumably these achromatic interchromomeric bands would continue to contract even after the chromomeres had come so close together that the interchromomeric regions were no longer clearly visible.

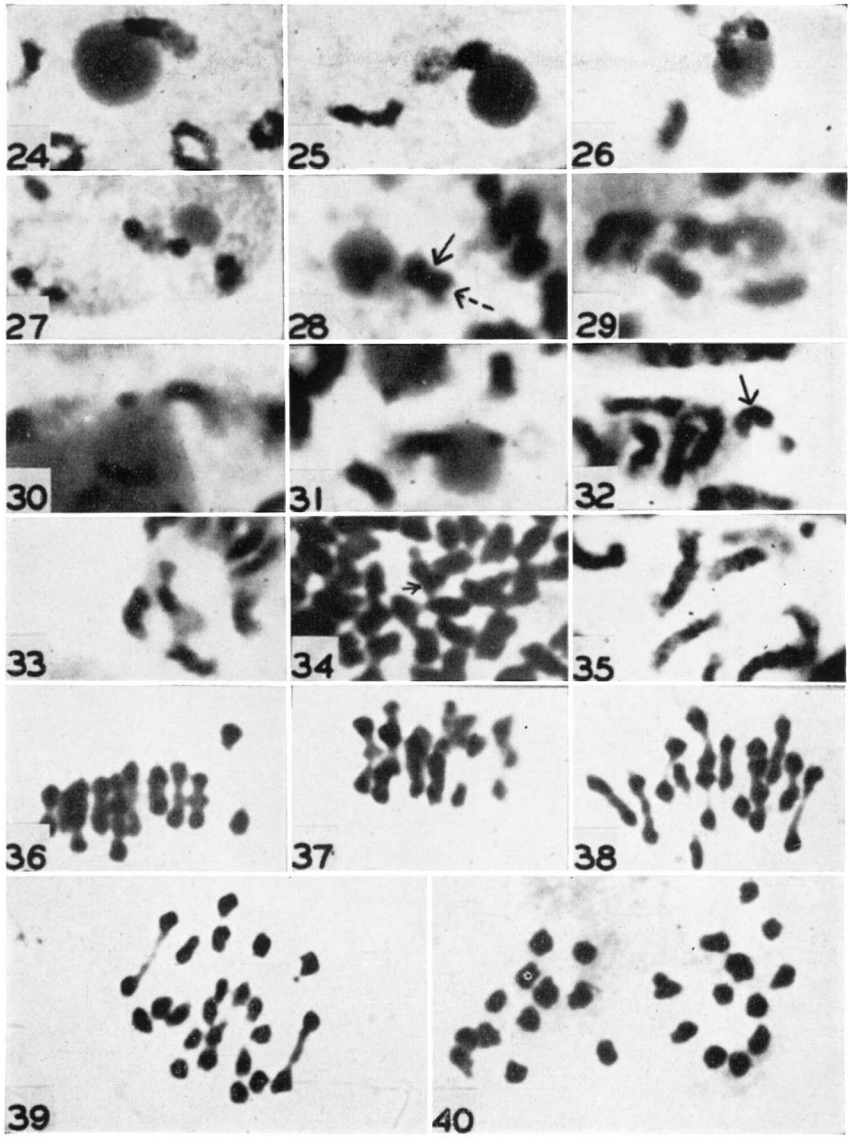

PLATE III

Early diakinesis to interphase, and mitotic stages. Figs. 28–33, 35, 3000×, others 2000×. Figs. 24–25. Nucleolus-organizing chromosome at middle diakinesis. Figs. 26–27. Nucleolus-organizing chromosome at late diakinesis. Figs. 28–29. Nucleolus-organizing chromosome at interphase. In fig. 28, straight arrow points to centromere constriction, broken arrow to longitudinal split. Figs. 30–31. Nucleolus-organizing chromosome with nucleoli at mitotic midprophase. Figs. 32–33. Nucleolus-organizing chromosome at late mitotic prophase. Arrow points to centromere constriction in fig. 32. Note the achromatic tail in fig. 33. Fig. 34. Nucleolus-organizing chromosome at mitotic metaphase. Arrow points to centromere constriction. Fig. 35. Chromosome at mitotic midprophase. Small achromatic region and telochromomeres are visible on the lower end. Fig. 36. Meiotic metaphase. Second and third bivalents from right have interstitial chiasmata. Fig. 37. Meiotic metaphase. Bivalent at left has large interstitial chiasma. Fig. 38. Early meiotic anaphase with achromatic zones between most of the separating bivalents. Interstitial chiasmata in the first and second bivalents from the left. Fig. 39. Early meiotic anaphase. Achromatic zones disappear as the bivalents complete their separation. Fig. 40. Late meiotic anaphase. Chromosomes show only chromatic material with exception of small achromatic ends of two chromosomes at the pole to the left.

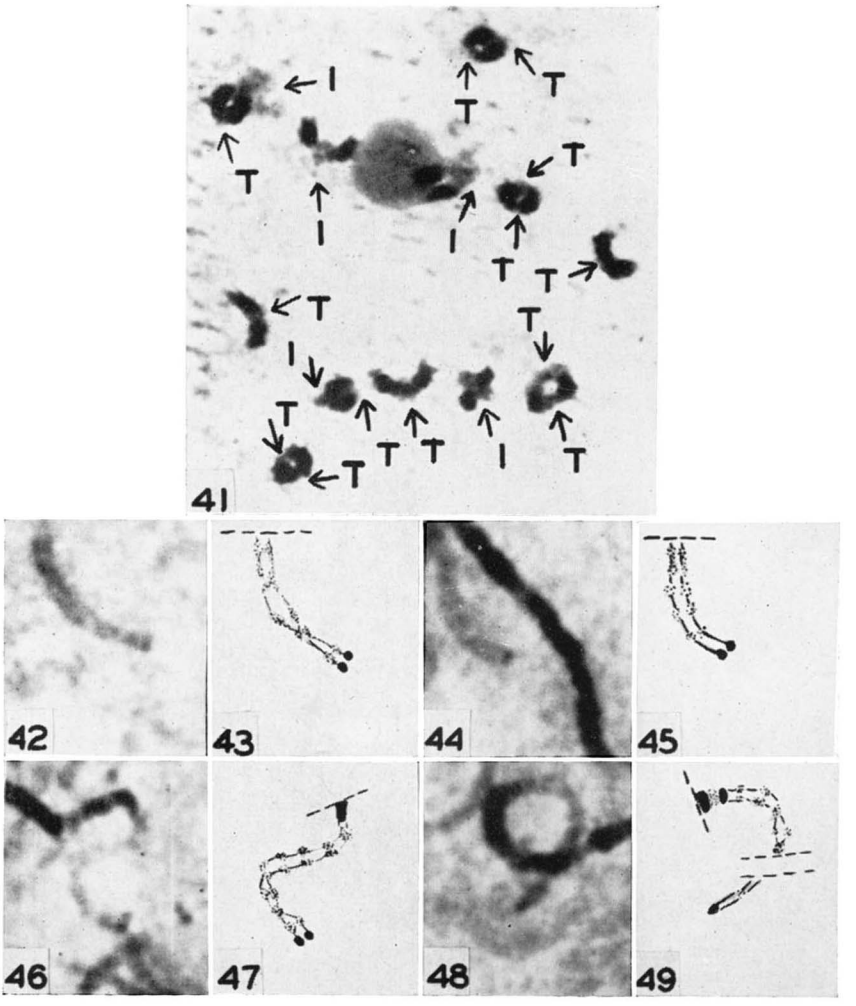

PLATE IV

FIGURE 41.—Diakinesis. 2000×. T, terminal chiasmata. I, interstitial chiasmata.

FIGURES 42–49.—Pachytene. 4000×. Figs. 42, 44, 46, 48. Photomicrographs showing delicate chromomere pattern in achromatic regions. Figs. 43, 45, 47, 49. Companion drawings, respectively, of the chromomere patterns.

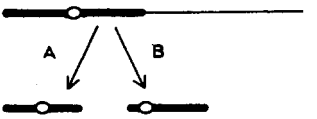

FIGURE 50.—Diagram illustrating the two possible results from different types of prophase behavior. If the achromatic material contracted to an extremely short length while the chromatic material contracted much less, a symmetric chromosome could result from an asymmetric one (arrow A). If the achromatic material became chromatized, the resultant chromosome would also be asymmetric (arrow B).

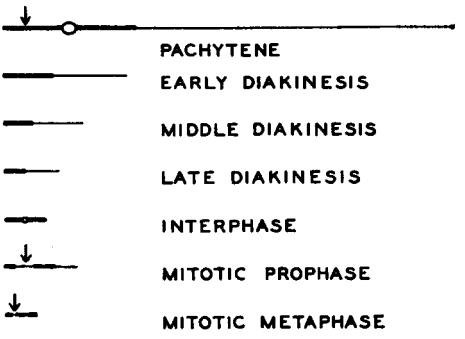

FIGURE 51.—Diagram based on measurements of lengths of various regions of the nucleolus-organizing chromosome during meiosis and mitosis. The vertical arrow at pachytene represents approximate region of attachment to the nucleolus. The broken line at mitotic stages (underneath arrows) represents the region where nucleolus is or has been. The centromere is represented as an oval in meiosis, and as a constriction in mitosis; it is not detectable during diakinesis.

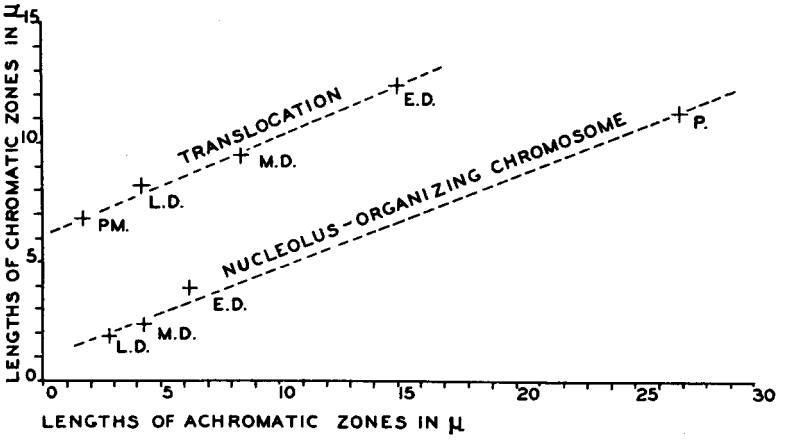

FIGURE 52.—Plots of the total lengths of the chromatic zones and the achromatic zones for the translocation ring-of-four chromosomes and the nucleolus-organizing chromosomes, P., pachytene; E.D., early diakinesis; M.D., middle diakinesis; L.D., late diakinesis, PM., prometaphase. The points represent averages for 15–25 measurements for the nucleolus-organizing chromosomes, and 25 measurements for the translocation rings. The straight lines were fitted to these averages by means of least squares; $y=6.09+0.41x$, for the translocation, and $y=1.03+0.37x$, for the nucleolus-organizing chromosome; y, length of chromatic zone; x, length of achromatic zone.

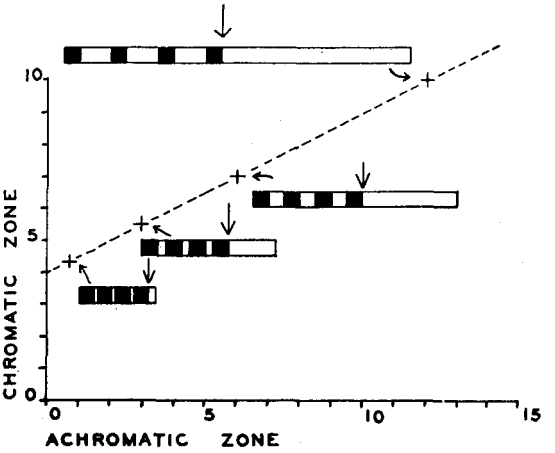

FIGURE 53.—Diagram illustrating an hypothesis of chromosome contraction. The horizontal bars represent chromosomes divided into chromatic zones, left of straight arrows, and achromatic zones, right of arrows. The chromatic zones are in turn divided into chromomeres, the black squares, and achromatic interchromomeric regions. The successively shorter bars represent chromosomes contracting. The achromatic, interchromomeric bands are assumed to contract at the same rate as the achromatic regions. The chromatic chromomeres are assumed not to contract. When the length of the entire chromatic zone is plotted against the length of the achromatic zone, a straight line which does not pass through the origin is obtained. No attempt has been made to illustrate coiling. Obviously, even a small amount of coiling would obscure the interchromomeric material in actual chromosomes.

DISCUSSION

The structure of the meiotic chromosomes of tomato resembles most closely those which have been regarded as differentiated into euchromatin and heterochromatin. The term, heterochromatin, was originally used by HEITZ (1929) to designate chromosomal material which maintained its metaphase stainability longer during telophase and acquired it earlier during prophase than the rest of the complement. In this report, the achromatic and chromatic zones of the tomato chromosomes have not been referred to as euchromatin and heterochromatin, respectively, for two reasons. The achromatic zones seem to shorten markedly without acquiring an appreciable capacity to stain darkly. Therefore they differ from the typical euchromatin, originally described by HEITZ, which does acquire during prophase a marked capacity for deep staining. Secondly, the various chromatic zones seem to show a differential behavior during the resting stage. While some apparently lose their stainability, others seem to clump together to form a chromocenter. Although much more study is needed to determine the exact process of chromocenter formation, it seems unwise to call all of the chromatic zones heterochromatin until their behavior in mitotic telophase nuclei can be further studied. In order to compare the behavior of the tomato chromosomes with that of other differentiated chromosomes, the achromatic and chromatic regions will be regarded as similar in some respects, if not identical to, eu- and heterochromatin.

Chromosome structure. At pachytene, the tomato chromosome may easily

be seen to consist of a centromere, proximal chromatic regions, distal achromatic zones, and telochromomeres. The morphology of the centromere is typical; its behavior in pairing will be considered later.

The chromatic zones are of interest in showing specific patterns of chromomeres. Chromomeres have been observed in heterochromatin in both meiotic and mitotic divisions. For the latter, DOBZHANSKY (1944) has pictured chromomeres of heteropycnotic regions in both the X and Y chromosomes of *Drosophila pallidipennis*. In plants, the meiotic chromosomes of Pellia (JACHIMSKY 1935) and Agapanthus and Kniphofia (DARLINGTON 1933) possess chromomeric heterochromatin. The B chromosome of maize is partially heterochromatic, and has a specific structure in the pycnotic regions (MCCLINTOCK 1933). It thus seems established that the heterochromatic regions may possess a chromomere pattern as specific as any found in euchromatic regions. In fact, in tomato the chromatic zones possess the obvious chromomere patterns, while those of the achromatic zones are much more difficult to distinguish.

DARLINGTON (1937) has discussed a type of heteropycnosis which appears near the centromere during meiosis, but not mitosis, in Agapanthus, *Zea mays* (the regular complement), Oenothera, and Canna. Because it appears in meiosis but not in mitosis, "It can, therefore, be represented simply as a timing difference at meiosis, the proximal parts being in advance of the distal parts. Moreover, since it applies to all chromosomes regularly in exactly corresponding regions it can scarcely be due to a differentiation of the genetic properties of the chromomeres; it must be due to the position of the precocious parts in relation to the centromere." (pp. 308–309, l. c.). Such is not the case in tomato where the chromomere patterns of the chromatic zones are variable from chromosome to chromosome among the complement, and constant for a particular chromosome. The possible effect of proximity to the centromere on the development of large amounts of stainable material can not be determined without experimental work.

Chromosome ends have frequently been associated with heterochromatin or heterochromatin-like structures or functions. Deep-staining chromosome ends have been observed at pachytene in Sphaerocarpus by LORBEER (1934). In *D. melanogaster*, tips of the salivary gland chromosomes frequently become fused with each other in the non-homologous fashion characteristic of the heterochromatin of these cells (PROKOFYEVA-BELGOVSKAYA 1937; HINTON and ATWOOD 1941). MULLER (1940) has postulated the existence of stable terminal ends of chromosomes, called "telogenes" or "telomeres." When a chromosome loses the telomere, it becomes labile and free to join with other such broken ends. Whether or not the telochromomeres have the role of making stable ends cannot be told by simple observational methods. Their seemingly typical, if not universal, presence in tomato indicates that they probably have some function, but this reasoning can not be extended to reveal what the function might be.

Chromosome pairing. In tomato, the centromeres may be observed to pair before the adjacent chromatic zones. Differential pairing behavior has been

reported for the centromere in Paris (HAGA 1944), and *Trillium kamtschaticum* (MATSUURA 1946). In these cases, some or all of the centromeres may remain in synaptic union until the first meiotic metaphase. In *Zea mays*, DARLINGTON (1934) noticed that the homologous centromeres are still paired until diplotene. In the tomato, the differential activity of the centromeres is apparent only because of the marked contrast with the pairing of the chromatic zones. Centromere pairing seems to be about synchronous with the pairing of the achromatic arms, and has completely disappeared by early diakinesis. Unfortunately the diplotene stage could not be studied to determine whether the centromeres separated more slowly than the other regions of the chromosome.

The slower pairing of the chromatic zones seems similar to pairing disturbances associated with heterochromatin. Different types of peculiar pairing behavior of the sex chromosomes of animals have been discovered in abundance (see WHITE 1945). In liverworts LORBEER (1934, 1936) observed a "distance" conjugation of the totally heteropycnotic sex chromosomes, and pairing in only the euchromatic regions of partially heteropycnotic chromosomes. On the contrary, MARQUARDT (1937) found that the heterochromatic regions of Oenothera paired very early in leptotene, while the euchromatin did not pair until zygotene. In Fritillaria and in Paris (DARLINGTON 1935, 1941, resp.) pairing was, in the first case, observed to begin in the proximal heterochromatic regions and proceed distally. In the second, it was believed to do so on the basis of localization of chiasmata.

Chiasmata. According to DARLINGTON (1937) tomato belongs to a class of organisms with completely terminalized chiasmata. UPCOTT (1935), and MACARTHUR and CHIASSON (1947) have both reported interstitial chiasmata at diplotene but claimed almost complete terminalization by diakinesis. On the other hand, AFIFY (1933) reported a terminalization coefficient of only 0.84 for diakinesis, and observed a retardation of bivalent separation at metaphase due to interstitial chiasmata. Although he was apparently unaware of the structural differentiation of the tomato chromosome, AFIFY'S results are more nearly comparable to the present findings than are those of the other workers. The apparent reason for the difficulty in seeing the interstitial chiasmata of the tomato at diakinesis is that they seem to occur in the achromatic regions. Fixing and staining techniques which do not reveal the proper structure of the achromatic zones would lead one to believe that the bivalents (that is, chromatic zones) are held together by fine threads pulled out by terminalized chiasmata.

In other plants, chiasmata have been found in heterochromatic regions with a much lower frequency than in euchromatic regions, if at all. In Oenothera MARQUARDT (1937) found no chiasmata in the heterochromatic regions and JAPHA (1939) found only a few rare exceptions to this rule. In Agapanthus GEITLER (1933) found as many as four chiasmata per bivalent, all in the euchromatic regions. MATHER (1939) has concluded from statistical studies that interstitial chiasmata are formed where they are found, and that terminalization is an all-or-none process. If his idea is true also for differentiated chro-

mosomes, chiasmata are not produced, or produced only infrequently in heterochromatic zones, and their occurrence outside such zones can not be simply attributed to terminalization processes.

Chromosome contraction. JAPHA (1939) reported that the euchromatin of Oenothera contracted to 1/25 of its original length from pachytene to metaphase while the heterochromatic regions contracted to only 1/5. She did not disprove the possibility of a conversion of the euchromatin to a heterochromatin-like appearance. She believed that the differential rate was attributable to the fact that the heterochromatic material had already become strongly spiralized at pachytene. If contraction of chromatic regions of tomato were merely precocious, rather than blocked, the straight line relationships for their relative rates would pass through the origin, which they do not do.

Although spiral structure can occasionally be seen in the tomato chromosomes during mitosis, it is not necessary to assume that chromosome contraction in the tomato is attributable to spiralization, or to any other particular mechanism, to account for the hypothesis advanced in the preceding section. Spiralization has been found in such a wide variety of organisms (see review by SERRA 1947) that it is believed it will eventually be demonstrated in meiosis of tomato. Conventional techniques for revealing spiral structure have so far resulted in a complete disorganization of the chromosomes and an intense darkening of the cytoplasm.

By the technique of absorption of ultra-violet light, CASPERSSON (1938) found that the amount of nucleic acid in meiotic nuclei increased until leptotene but showed no further change as far as diplotene when the measurements were terminated. Because most of the chromosome contraction occurred after leptotene, CASPERSSON concluded that an increase in gross amount of nucleic acid was not necessary for contraction. He admitted that the nucleic acid might play a secondary role in chromosome contraction, but pointed out that if nucleic acid were responsible for chromosome contraction it should be present in the bands between the leptotene chromomeres. His study showed that nucleic acid was absent from the interchromomeric bands at leptotene just as it was absent from the material between the chromatic bands of the salivary gland chromosomes.

The material examined in the tomato is chromosomal substance differing in degree of stainability with aceto-carmine, and consequently the results of this study can not be compared directly with quantitative determinations of nucleic acid content. MIRSKY and RIS (1947a, b) have recently shown that a peripheral material which is responsible for chromosome staining may be removed leaving a residual strand which is much less chromatic. The peripheral material contained the bulk of the nucleoprotein of the chromosome.

Even if this suggestion of the greater nucleoprotein content of the tomato chromatic zones were to be discounted, there is still a remarkable similarity between the conclusions reached by CASPERSSON in his study of the amounts of nucleic acid and those reached here by a study of chromosome regions differing in amounts of stainable material. In both cases, an increase in amount of

nucleic acid, or of stainable material, does not seem to be necessary for chromosome contraction. Yet in both cases a very small amount may be all that is required, and this small amount may, after it is once acquired in early prophase, produce its effect through changes in its relationship with other components of the chromosome rather than by changes in amount.

SUMMARY

At pachytene, the chromosomes of tomato possess a highly differentiated structure. The centromere is surrounded on both sides by chromatic zones with specific chromomere patterns. The distal ends are achromatic and have a very delicate pattern of chromomeres which are difficult to detect. The achromatic regions form at least half the length of all chromosomes, and in some cases, much more than half. The ends of the chromosomes are typically terminated by distinct chromatic chromomeres, the telochromomeres.

The chromatic and achromatic zones are in some ways similar to the hetero- and euchromatin, respectively, of other organisms.

The chromatic zones pair at zygotene and early pachytene at a slower rate than do either the centromeres or the achromatic zones.

In the material examined, there was an average of 1.33 chiasmata per bivalent, and a terminalization coefficient at diakinesis of 0.70. In the long arm of the nucleolus-organizing chromosome, chiasmata were only 30 percent terminalized. In the completely chromatic short arm of the nucleolus-organizing chromosome, chiasmata occurred at a significantly lower frequency than would be expected from the pachytene length of this arm, if they occurred at all.

The achromatic zones contributed very little to the length of the contracted chromosome. This was demonstrated by following the reduction in length during meiotic prophase of an asymmetric chromosome with a long achromatic end.

The chromatic zones shorten at a rate much slower than that of the achromatic zones. The relative plots show a straight line relationship which does not pass through the origin. This may be most easily explained on the basis that the chromatic zones contain interchromomere regions which contract at the same rate as the distal achromatic zones while the chromatic chromomeres remain resistant to contraction.

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