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STRUCTURE OF AND HOMOLOGY BETWEEN PACHYTENE AND SOMATIC METAPHASE CHROMOSOMES OF THE TOMATO *)

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The pachytene and somatic metaphase chromosomes of tomato are structurally differentiated into proximal chromatic and distal achromatic parts. The pachytene chromosomes have very clear and characteristic chromosome markers, with the help of which all 12 bivalents can be clearly identified. Based on the size, the arm ratio, the ratio of chromatic parts and the presence and size of achromatic parts, all 12 pairs of somatic chromosomes can also be identified, and each pair be homologised with the corresponding pachytene bivalent. A comparison of the lengths of chromatic and achromatic parts of pachytene chromosomes with the chromatic and achromatic parts of the corresponding somatic chromosomes indicate, that, on an average, the chromatic parts are contracted by a factor of 4 to 5, whereas the achromatic parts are contracted by a factor of 30. The heteropycnosis near the centromere in tomato chromosomes therefore is not a special characteristic of meiotic chromosomes, but present in somatic metaphase chromosomes also.

Introduction

Morphological differentiation of pachytene chromosomes of tomato, as revealed by their staining properties, has been a subject of several cytological investigations (LESLEY & LESLEY, 1935; BROWN, 1949; BARTON, 1950; GOTTSCHALK, 1951, 1958; LIMA-DE-FARIA & SARVELLA, 1962). The chromosomal differentiation into darkly staining *proximal* (adjacent to centromere) and lightly staining *distal* regions (away from the centromere) found in tomato represents a typical case of a widely occurring phenomenon in many plant genera (RICK & KHUSH, 1964). The proximal regions have generally been referred to as "centric heterochromatin", implying that their cytological and genetic be-

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haviour correspond to heterochromatin in other organisms, the distal parts being indicated as "euchromatic". Although the terms hetero- and euchromatin in the above context form a subject of controversy, the structural differentiation of pachytene chromosomes and the differential behaviour of their parts have been well recognised (BROWN 1949).

The comparison of meiotic and mitotic chromosome morphologies in *Agapanthus, Zea mays, Oenothera* and *Canna* has lead to the view, that the heteropycnosis near the centromere appears only during meiosis, not during mitosis. From this observation it is concluded that the heteropycnosis is due to timing difference at meiosis, the proximal parts being in advance of the distal parts with respect to spiralization (DARLINGTON, 1937). In tomato, as will be described in this article, the situation appears to be different.

In the present communication, observations on the structural differentiation of and homologies between pachytene and somatic metaphase chromosomes are reported. In addition, definite criteria for the identification of the 12 pachytene bivalents and also of the 12 pairs of somatic metaphase chromosomes are given.

Material and methods

Pachytene and somatic chromosomes were studied in an inbred line of *Lycopersicum esculentum* MILL, variety Moneymaker. For comparison, two other varieties, Glory-m and Artresist, obtained by the courtesy of Ir. E. KOOISTRA, were used.

For the study of pachytene chromosomes, the flower buds were collected from plants grown in the greenhouse. Anthers were fixed in a freshly prepared solution of 1 part of propionic acid saturated with iron acetate and 3 parts of absolute alcohol, for 48 hrs or more at room temperature. When anthers were black in colour, which seems to be a good indication of proper mordanting, they were squashed in a drop of 1% aceto carmine. Judicious heating of the slides, after squashing, greatly helps in spreading the pachytene chromosomes.

Mitotic chromosomes were studied in root tips from seeds germinated at room temperature up to a length of nearly $\frac{3}{4}$ cm. Before fixation, germinating seeds were treated with a saturated aqueous solution of α-bromonapthalene for 90 minutes. Fixations were made in acetic

alcohol 1 : 3 for 48 hrs at room temperature. Roots were hydrolysed in 1N hydrochloric acid for 7 minutes at 59°C in a water bath. After hydrolysis, they were washed in 70% alcohol for 2 minutes, followed by water for 1 minute, stained with Feulgen reagent for 2 hours and squashed in a drop of 1% aceto carmine.

The slides were made permanent by separating the cover glasses in a solution of 1 : 1 acetic acid and n-butyl alcohol and, after a change in pure n-butyl alcohol, mounting in euparol.

All observations were carried out using a phase contrast microscope. Pachytene chromosomes were measured from their drawing, made with the use of a Camera Lucida attachment to a Wild M20 microscope, using normal illumination. The length of each individual pachytene chromosome was determined as the average value of not less than 25 measured chromosomes. For all measurements and the study of morphological details, cells in mid pachytene stage were selected, since this was the best stage for the accurate identification of chromosomes. In 15 cells nearly all 12 bivalents could be traced and measured from one end to the other. The remaining measurements were made from cells selected for good chromosomes.

Pachytene chromosomes are numbered in accordance with BARTON (1950), who had followed the method of numbering adopted by MCCLINTOCK (1929) in *Zea mays*. According to this, the longest chromosome has been indicated as chromosome 1, and the successive ones as 2, 3 and so on. Morphological details were recorded accurately from ideal preparations and depicted in the idiogram after a great number of comparisons. Although the scheme presented in Figure 2 is a rather idealised one, the structural details represented can be well traced in fairly good preparations. Only those structural features which could be invariably traced are given as identification markers for pachytene chromosomes, and indicated by small letters (a, b, c etc.) in Figure 2. Compare also the photographs 1a and 1b.

Somatic chromosomes were measured with a Camera Lucida, according to the procedure developed by SYBENGA (1964). The length of each chromosome was determined as the average of the lengths of the chromosome concerned in 15 cells, i.e. the average of 30 chromosomes.

Terminology: The chromosome regions which stain darkly and lightly are termed "chromatic" and "achromatic" parts respectively, as was

done by BROWN (1949). The long and short arms of the chromosomes are indicated by L and S respectively, followed by the particular chromosome code number. For example, 12 L and 12 S indicate the long and the short arm of chromosome 12. Long arms have been drawn on the lower side of the centromere in the idiogram (Fig. 2).

Results

STRUCTURE OF PACHYTENE CHROMOSOMES

Four types of general characteristic structures can be recognised in all 12 bivalents. They are, 1) centromere, 2) chromatic parts, 3) achromatic parts and 4) telomeres (Figures 1a and 1b). On both sides of the centromere, the chromosome arms are differentiated into a proximal chromatic and a distal achromatic region, with the exception of 2 S having no achromatic part.

Centromeres are clearly visible as short or more elongated achromatic structures. No chromatic elements are visible in the centromere regions.

Each chromatic part consists of darkly staining chromomeres, often with very short achromatic segments in between them. Within a chromatic part, the chromomeres differ from each other in size and staining intensity in a rather constant way, and each region has a distinct and characteristic organization of chromomeres. The exact number of chromomeres is, however, difficult to represent in Figure 2, as in the course of pachytene individual chromomeres seem to fuse with each other. In specific positions of some of the chromatic parts interchromomeric segments are constantly large or conspicuous, and such segments are referred to as "gaps" in the following sections of the text. Such gaps are highly characteristic for some of the chromatic parts (see next section).

In each arm (except 2 S) the chromatic part is followed by the achromatic part. These achromatic parts are visible as lightly staining and almost homogeneous extensions. They usually lack clearly defined chromomeric structures, especially in the distal regions. In the portion immediately adjacent to the chromatic part of the long or the short arm of some of the chromosomes, small lightly staining chromomeres may be seen, and these appear as transition zones

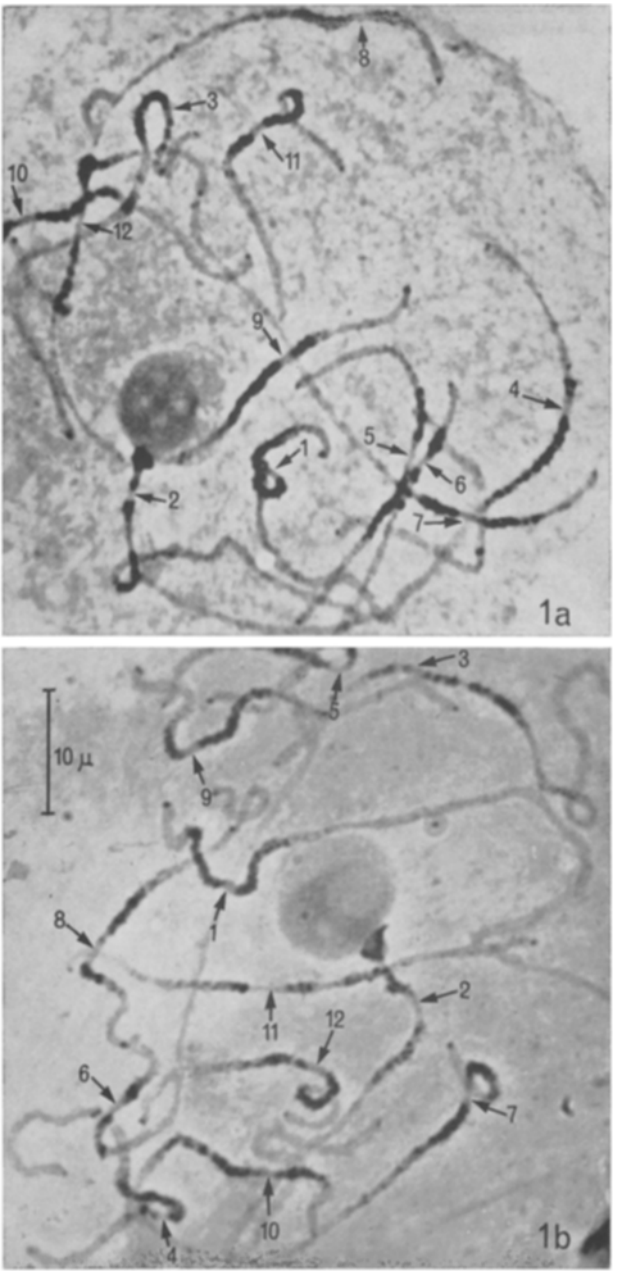


Figure 1a and b. Photomicrographs of pachytene chromosomes of tomato Arrows indicate the centromeres of chromosomes 1–12.

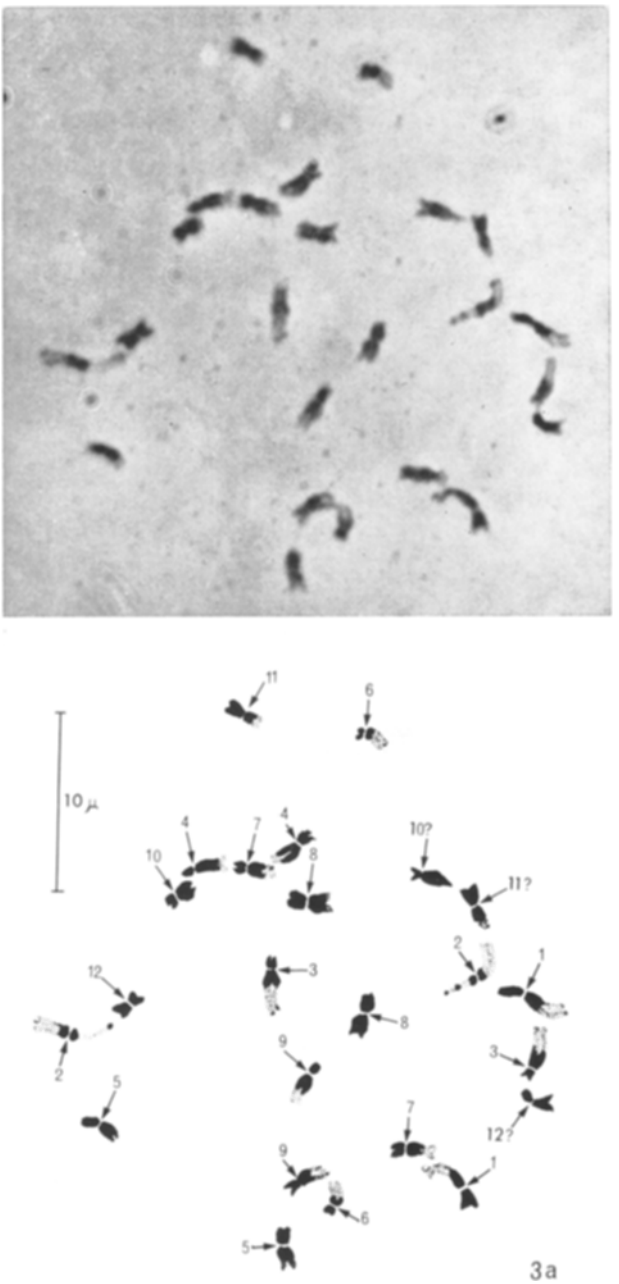
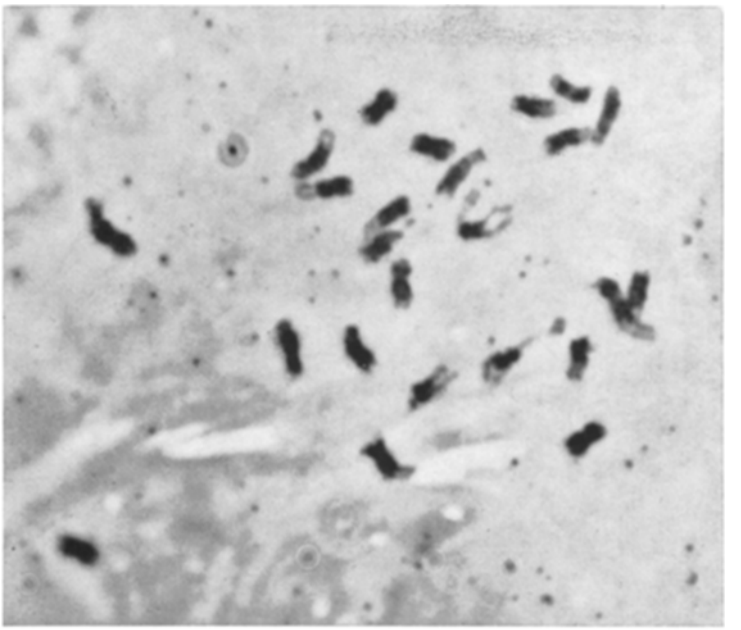


Figure 3a and b. Photomictrographs and drawings of somatic metaphase chromosomes of tomato. Individual chromosome pairs numbered in the order corresponding to 1 to 12 of pachytene bivalents. Arrows indicate centromeres.



3b

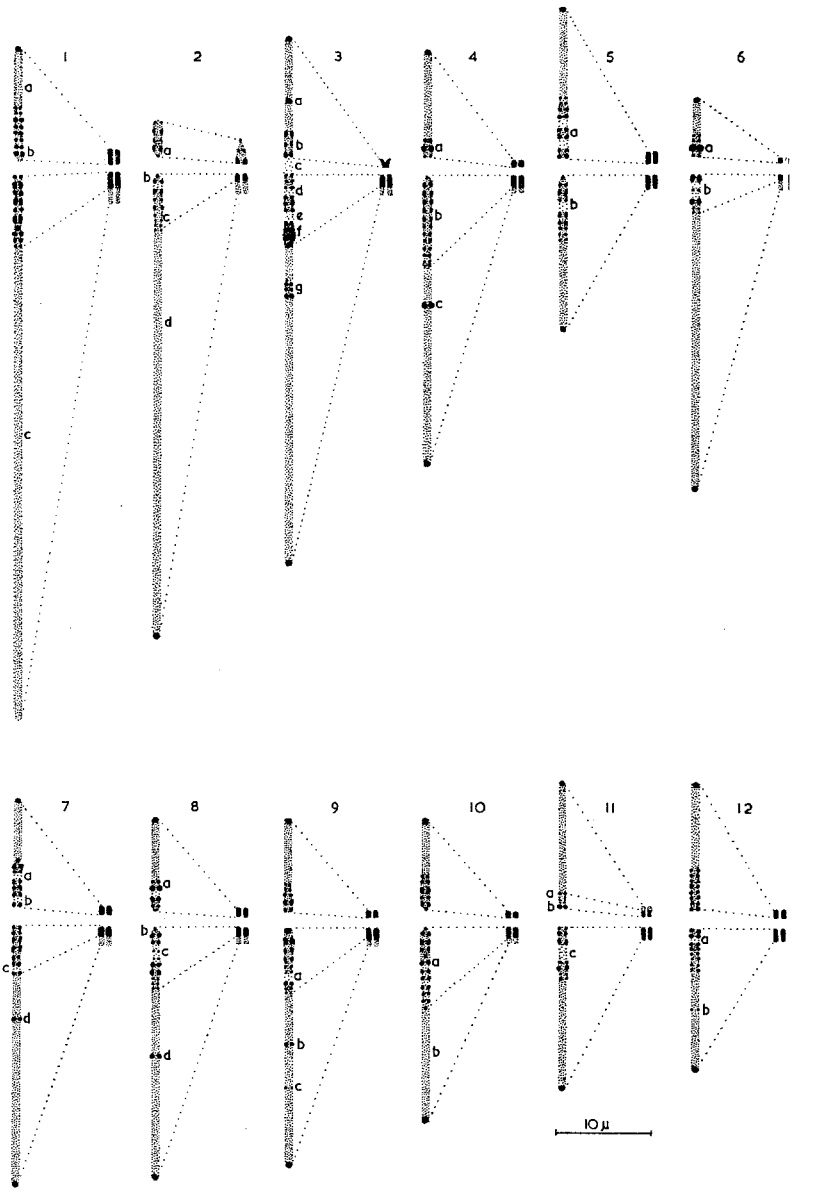


Figure 2. Idiograms of pachytene and somatic metaphase chromosomes of tomato, and the comparison of the lengths of chromatic and achromatic parts during the two stages. Identification markers of pachytene chromosomes indicated by a, b, c etc. for each chromosome. Arms of somatic chromosomes pictured quite seperately, while actually centromere only visible as a slight constriction.

between the chromatic and achromatic parts (e.g. 2 L, 4 L, 5 L and 5 S, 1 S, 11 S). A single, isolated and darkly staining chromomere, or a small group of chromomeres, is observed in the achromatic regions of some of the chromosomes (e.g. 3 L and 3 S, 4 L, 8 L, 9 L, Figure 2). These isolated chromomeres are very useful for the identification of chromosomes.

The chromosome arms end in darkly staining telomeres which do not vary greatly in size in different chromosomes. In 1 L, the telomere is obscure and in 2 S it is indistinguishable because of the absence of an achromatic part. The above observations are true for all three varieties studied.

IDENTIFICATION OF PACHYTENE BIVALENTS

The following three criteria should be kept in mind when identifying the pachytene chromosomes. 1) The total length and arm ratio's of chromosomes, 2) The proportion of chromatic parts on both arms and 3) The characteristic chromomeres and gaps that have identification value.

From the comparison of the average lengths of chromosomes 1 and 12 (table 1), it may be observed that chromosome 1 is approximately 2.4 times as long as chromosome 12. Such ratio of length of any two different chromosomes is rather variable in different cells. Obviously, this is due to the highly variable lengths of individual chromosomes in different cells, partly depending upon differences in contraction and/or stage and partly upon variable stretching during fixation and spreading. In spite of such variation in length, the chromosomes may, for the sake of convenience, be categorised into long, medium and short types. Chromosomes 1, 2 and 3 exceed an average length of 50μ and are classified as long chromosomes. Chromosomes 4, 6, 7, 8 and 9, which have a length between 42 and 35μ, are classified as medium ones. The remaining four chromosomes, 5, 10, 11 and 12, which are between 38 and 32μ, belong to the group of short chromosomes.

The ratio of the long and short arm of a particular chromosome is also variable in different cells. Nevertheless, they can be easily classified as asymmetric (total length of the long arm being at least twice that of the short arm) and symmetric (the length of the arms being nearly equal). All of the eight long and medium chromosomes

TABLE 1

AVERAGE LENGTH OF CHROMATIC AND ACHROMATIC PARTS AND OF ENTIRE PACHYTENE CHROMOSOMES, IN MICRONS, 25 MEASUREMENTS FOR EACH

	chromosome	long arm		short arm		total	ratio
		mean	s.d.	mean	s.d.		chromatic parts
1	chromatic	6.3	0.73	5.5	0.73		1.15
	achromatic	49.8	8.00	6.3	1.18		
	total	56.1		11.8		67.9	
2	chromatic	5.1	1.68	4. 4	1.00		1.16
	achromatic	42.9	8.36	—	—		
	total	48.0		4.4		52.4	
3	chromatic	6.6	1.18	2.5	1.64		2.64
	achromatic	33.6	5.50	10.2	2.55		
	total	40.0		12.7		52.9	
4	chromatic	8.4	1.00	1.7	0.04		4.94
	achromatic	21.5	7.00	9.3	2.00		
	total	29.5		11.0		40.5	
5	chromatic	6.0	0.86	4.7	0.73		1.28
	achromatic	10.7	2.32	10.4	2.91		
	total	16.7		15.1		31.8	
6	chromatic	3.5	0.50	1.8	0.04		1.94
	achromatic	29.5	5.18	4.5	0.77		
	total	33.0		6.3		39.3	
7	chromatic	5.6	1.27	4.5	0.73		1.24
	achromatic	20.9	5.55	6.9	1.91		
	total	26.5		11.4		37.9	
8	chromatic	4.8	0.55	2.8	0.55		1.71
	achromatic	21.3	4.00	6.8	1.73		
	total	26.1		9.6		35.7	
9	chromatic	7.2	1.18	3.3	0.55		2.18
	achromatic	17.1	4.14	9.0	2.14		
	total	24.3		12 .3		36.6	
10	chromatic	9.4	1.50	3.0	0.68		3.13
	achromatic	11.3	2.27	6.1	1.00		
	total	20.7		9.1		29.8	
11	chromatic	6.0	0.73	2.2	0.04		2.72
	achromatic	10.7	1.87	11.1	2.32		
	total	16.7		13.3		30.	
12	chromatic	5.6	0.95	4.8	1.00		1.16
	achromatic	9.3	2.14	8.8	1.86		
	total	14.9		13.6		28.5	

are asymmetric, whereas only one of the four short chromosomes, i.e. chromosome 10, is asymmetric, while 5, 11 and 12 are symmetric.

The ratio of the chromatic part on the long and that on the short arm is highly characteristic for each chromosome. Depending on this, the chromosomes can also be classified into two categories: 1) Chromosomes with asymmetric chromatic parts and 2) Chromosomes with symmetric chromatic parts. Out of the nine asymmetric bivalents, five have symmetric chromatic parts (1, 2, 6, 7 and 8) and four have asymmetric chromatic parts (3, 4, 9 and 10). Among the three symmetric bivalents, two have symmetric (5, 12) and one has asymmetric chromatic parts (11).

Although the total length, the symmetry of the arms and the symmetry of the chromatic parts are useful for a broad classification of all pachytene bivalents, they do not offer an absolute criterion for the identification of all 12 bivalents. This can be achieved, however, with the help of characteristic chromomeres and gaps, which are useful as identification markers for each of the chromosomes. In many cases, regardless of the length of the arms and the length and symmetry of the chromatic parts, the pachytene bivalents can be identified with the use of such markers alone. Of the numerous characteristics, many correspond with those described by BARTON (1950).

In the following description only the features with high identification value will be mentioned. The special markers are indicated a, b, c etc., which corresponds with the indications in Figure 2.

Chromosome 1.

Longest by far. Asymmetric. Chromatic parts symmetric.

a. In achromatic part of 1 S very small, light chromomeres.

b. At proximal beginning 1 S dark, prominent chromomere.

c. Achromatic part 1 L without chromomeres. Telomere obscure.

Chromosome 2.

Long. Nucleolus in 2 S. Asymmetric. Chromatic parts nearly symmetric. Localisation centromere generally difficult, aided by a., b.

a. At proximal beginning 2 S (chromatic part) very small chromomere followed by massive one.

b. At proximal beginning 2 L (chromatic part) very small chromomere followed by larger one, followed by clear gap. Gap sometimes greatly extended, resembling centromere.

c. Distal region chromatic part 2 L without larger chromomeres.

d. Achromatic part 2 L without chromomeres except telomere.

Chromosome 3.

Long. Asymmetric. Chromatic parts highly asymmetric.

a. At $^1/_3$ achromatic part 3 S clear chromomere.

b. Chromatic part 3 S consisting of three small dots.

c. Centromere unclear.

d., e. In chromatic part 3 L two faint gaps.

f. At distal end chromatic part 3 L row of large dark chromomeres.

g. At one sixth achromatic part 3 L cluster of large and small chromomeres.

Chromosome 4.

Medium length. Asymmetric. Chromatic parts highly asymmetric.

a. Chromatic part 4 S consisting of one large chromomere with small one on either side.

a. In chromatic part 4 L chromomeres clearly separated.

b. In achromatic part 4 L one prominent, dark chromomere.

Chromosome 5.

Short. Symmetric. Chromatic parts asymmetric. Distinction from chromosome 12 difficult. Distinction between arms difficult. Chromatic part 5 S slightly shorter than 5 L.

a. In chromatic part 5 S two or more gaps.

b. In chromatic part 5 L clear gap.

According to the present observations this chromosome is not the fifth in length, but shorter. Since all other chromosomes (including 12, also based on a study of triplo 12) could with certainty be identified with the description of BARTON (1950) this chromosome was concluded to be the same as BARTON's chromosome 5. To avoid confusion, the order has been maintained as proposed by BARTON.

Chromosome 6.

Medium length. Asymmetric. Chromatic parts symmetric. Shortest chromatic region of entire complement.

a. Chromatic part 6 S consisting of one prominent, dark chromomere with one medium chromomere proximally, two small ones distally.

b. Chromatic part 6 L consisting of two large chromomeres, each sometimes appearing double. Small, clear chromomere in between the large ones.

Chromosome 7.

Medium length (approximately equal to 8 and 9). Asymmetric. Chromatic parts nearly symmetric.

a. Near distal end chromatic part 7 S clear gap, followed by 2–3 chromomeres.

b. In proximal part 7 S (chromatic part) clear gap.

c. At distal end chromatic part 7 L clear chromomere, preceded by gap.

d. At $^1/_4$ of achromatic part 7 L faint chromomere.

Chromosome 8.

Medium length (approximately equal to 7 and 9). Asymmetric. Chromatic parts nearly symmetric.

a. At distal end chromatic part 8 S gap followed by dark chromomere(s).

b. At proximal beginning chromatic part 8 L small, clear chromomere.

c. In center chromatic part 8 L prominent gap with few small chromomeres inside.

d. In center achromatic part 8 L dark chromomere.

Chromosome 9.

Medium length (approximately equal to 7 and 8). Asymmetric. Chromatic parts asymmetric.

a. In distal portion chromatic part 9 L clear gap.

b., c. In achromatic part 9 L two small chromomeres.

Chromosome 10.

Short. Asymmetric. Chromatic parts asymmetric.

a. In chromatic part 10 L chromomeres evenly spaced with small intervals. Chromomere size decreasing from proximal to distal.

b. Achromatic part 10 L relatively short ($^1/_2$ of 10 L), without chromomeres except telomere.

Chromosome 11.

Short. Symmetric. Chromatic parts asymmetric. Identification easy.

a. At distal end short chromatic part 11 S a distinct chromomere preceded by two or three small ones.

b. At proximal end of 11 S large, dark chromomere.

c. In chromatic part 11 L clear, large gap, resembling centromere, but containing chromatic elements. Centromere further distinguished by location next to b.

Chromosome 12.

Short. Symmetric. Chromatic parts symmetric. Arms difficult to distinguish (compare 5).

a. At proximal end chromatic part 12 L two prominent chromomeres followed by clear gap.

b. In center achromatic part 12 L small chromomere.

The identification markers indicated above for the 12 bivalents are applicable to all three varieties studied. The only difference observed is with respect to 2 S, which is rather long in Glory-m and Artresist, whereas it is relatively short in Moneymaker.

STRUCTURE OF SOMATIC METAPHASE CHROMOSOMES

As in pachytene chromosomes, clear morphological differentiation is observed in somatic metaphase chromosomes. The three characteristic structures that can be observed in them are, 1) centromere, 2) chromatic parts and 3) achromatic parts. Telomeres are usually inconspicuous or quite invisible (Figure 3a and b).

Centromeres are clearly visible only at metaphase; they are not distinguishable at prophase or prometaphase. At metaphase they appear as a clear constriction.

The chromatic parts are always adjacent to the centromere and they appear as darkly staining undifferentiated regions, i.e. without any clearly defined chromomeres or gaps. The proportion of the chromatic parts on both arms is rather characteristic for each individual chromosome, and is of great value for the identification.

The achromatic parts are paler and narrower than the chromatic ones, and they are conspicuous in the long arms of all the asymmetric chromosomes (figs. 3a and b). In the short arms of all asymmetric chromosomes, and on both arms of the symmetric chromosomes, they are invisible. The only exception is chromosome 11, which shows a short achromatic part on the short arm.

The lengths of individual chromosomes and arms, together with the lengths of chromatic and achromatic parts are given in Table 2.

IDENTIFICATION OF SOMATIC METAPHASE CHROMOSOMES

Unlike the pachytene chromosomes, the somatic metaphase chromosomes are rather difficult to identify. With some care, however, it appeared possible to recognize almost all 12 individual chromosome pairs, on basis of total length, the ratio of long and short arms and the ratio of chromatic and achromatic parts. During this careful analysis, a close parallelism between the structure of pachytene and of somatic metaphase chromosomes became apparent, which made it possible to homologize the 12 somatic metaphase chromosome pairs with the pachytene chromosomes 1–12. Below, the somatic metaphase chromosomes are therefore described in comparison with the corresponding pachytene chromosomes (compare Figure 2).

Chromosome 1. The largest chromosome at pachytene, no. 1, is an

TABLE 2

AVERAGE LENGTH OF CHROMATIC AND ACHROMATIC PARTS AND OF ENTIRE SOMATIC METAPHASE CHROMOSOMES, IN MICRONS, 30 MEASUREMENTS OF EACH

	chromosome	long arm		short arm		total	ratio
		mean	s.d.	mean	s.d.		chromatic parts
1	chromatic	1.58	0.14	1.52	0.10		1.04
	achromatic	1.48	0.28	—	—		
	total	3.06		1.52		4.58	
2	chromatic	0.76	0.07	0.74	0.06		1.03
	achromatic	1.61	0.24	—	—		
	total	2.37		0.74		3.11	
3	chromatic	1.35	0.10	0.75	0.09		1.79
	achromatic	0.95	0.37	—	—		
	total	2.30		0.75		3.05	
4	chromatic	1.42	0.17	0.68	0.10		2.08
	achromatic	0.59	0.12	—	—		
	total	2.01		0.68		2.69	
5	chromatic	1.40	0.08	1.23	0.11		1.38
	achromatic	—	—	—	—		
	total	1.40		1.23		2.63	
6	chromatic	0.76	0.06	0.76	0.06		1.00
	achromatic	1.09	0.22	—	—		
	total	1.85		0.76		2.61	
7	chromatic	1.14	0.17	1.11	0.14		1.03
	achromatic	0.72	0.09	—	—		
	total	1.86		1.11		2.97	
8	chromatic	1.25	0.13	1.00	0.13		1.24
	achromatic	0.70	0.14	—	—		
	total	1.95		1.00		2.95	
9	chromatic	1.54	0.15	0.94	0.13		1.51
	achromatic	0.67	0.08	—	—		
	total	2.01		0.94		2.95	
10	chromatic	1.46	0.02	0.95	0.08		1.55
	achromatic	0.42	0.01	—	—		
	total	1.88		..95		2.83	
11	chromatic	1.52	0.12	0.75	0.72		2.02
	achromatic	—	—	0.44	0.11		
	total	1.52		1.19		2.71	
12	chromatic	1.14	0.10	1.00	0.05		1.14
	achromatic	—	—	—	—		
	total	1.14		1.00		2.14	

asymmetric chromosome with symmetric chromatic parts and a very long achromatic part in the long arm. The largest pair of the somatic metaphase chromosomes is also asymmetric, with symmetric chromatic parts and with one long achromatic part, which, however, is only slightly longer than the chromatic part of the arm.

Chromosome 2. The nucleolar chromosome. In somatic metaphase it is represented by a pair of large satellite chromosomes, also the second on basis of total length. As in chromosome 1, the centromere is in the centre of the chromatic part, while the long arm shows an achromatic part of almost the same length as in chromosome 1. The short arm bears terminally a small satellite, usually subdivided into two dots. In most cases the connection between satellite and proximal part of the short arm is so much extended that superficially chromosome 2 can appear as a more or less symmetric chromosome.

Chromosome 3. The third pachytene chromosome is asymmetric, with a faintly staining small chromatic region on 3 S and with a rather unclear centromere. This chromosome is apparently identical to a pair of somatic metaphase chromosomes (also the third in length) that is highly asymmetric, with a small, narrow and faintly straining short arm and an unclear centromere. In addition it has, like the pachytene chromosome, a moderately long achromatic part on the long arm.

Chromosome 4. The fourth pachytene chromosome, having a very short darkly staining chromatic part on the short arm and a long chromatic part on the long arm, clearly corresponds to the pair of somatic chromosomes with a very small and darkly staining short arm and a much longer long arm, the achromatic part in which is much smaller than in chromosomes 1, 2 and 3.

Chromosome 5. See chromosomes 11 and 12.

Chromosome 6. Pachytene chromosome 6 has the smallest chromatic parts of all 12 chromosomes, of about equal size in both arms, and it has a long achromatic part. It can be reasonably compared with the pair of somatic chromosomes having the shortest chromatic parts (almost symmetric), and a long achromatic part.

Chromosomes 7 and 8. Pachytene chromosomes 7 and 8 both have approximately symmetric chromatic parts, without much difference in size between the two chromosomes, while also the achromatic parts in both chromosomes have about equal length. These two pachytene

chromosomes correspond to two pairs of somatic chromosomes which also have nearly symmetric chromatic parts and which show a short achromatic part on the long arm. In somatic metaphase the two pairs cannot always be distinguished one from the other, although the 8th pair has slightly asymmetric chromatic parts.

Chromosomes 9 and 10. Pachytene chromosomes 9 and 10 are asymmetric with asymmetric chromatic parts. They correspond to two pairs of asymmetric somatic chromosomes which have asymmetric chromatic parts with a short achromatic part on the long arm. These two pairs of somatic chromosomes resemble each other to some extent, but chromosome 9 has the longer achromatic part.

Chromosomes 5, 11 and 12. Pachytene chromosomes 5, 11 and 12 are the only three symmetric chromosomes of the complement. Of these 5 and 12 have symmetric chromatic parts and resemble each other to a great extent, as the achromatic parts on the long and short arms of both chromosomes are also of about the same length. Chromosome 11 on the other hand has clearly asymmetric chromatic parts and it has a fairly long achromatic part attached to the short chromatic part on the short arm. Corresponding to these three symmetric pachytene chromosomes, there are three pairs of somatic metaphase chromosomes which are also symmetric. Of these, two pairs have symmetric chromatic parts and lack achromatic parts on both (long and short) arms, while one pair has asymmetric chromatic parts and a short achromatic part attached to the shorter chromatic part. The first two symmetric pairs, without achromatic parts, correspond to pachytene chromosomes 5 and 12 and the two pairs cannot be distinguished one from the other. The one symmetric pair with asymmetric chromatic parts and one short achromatic part, therefore corresponds with pachytene chromosome 11.

The comparisons made above seem to be certain for most of the chromosomes, but for a few they are to be corroborated by studying various trisomics.

LENGTH RELATIONSHIP BETWEEN PACHYTENE AND SOMATIC METAPHASE CHROMOSOMES

The degree of condensation of chromatic and achromatic parts of pachytene bivalents, by the time they reach first meiotic meta-

phase cannot be determined, due to the fact that not all individual bivalents can be identified at MI. Moreover the achromatic parts are not conspicuous. However, comparisons can be made with somatic metaphase chromosomes on a segment to segment basis, since the metaphase chromosomes can be identified and are differentiated into easily recognisable chromatic and achromatic parts.

From the comparison of the mean lengths of chromatic and achromatic parts of pachytene chromosomes and of the corresponding somatic metaphase chromosomes, it may be concluded (Table 3) that the chromatic parts on the average are reduced in length by a factor of 4–5, the achromatic parts by a factor of 25–30. Chromatic parts of 2 S and 2 L, 4 L and 10 L seem to have a greater condensation coefficient (Table 3) when compared to other chromosomes, whereas

TABLE 3

THE LENGTH RELATIONSHIP BETWEEN PACHYTENE AND SOMATIC METAPHASE CHROMOSOMES (CONTRACTION FACTORS)

	Achromatic Pachytene/somatic	Chromatic Pachytene/somatic	
		Long arm	Short arm
1	33.64	3.90	3.62
2	26.64	6.71	5.94
3	35.36	3.33	4.89
4	36.64	5.92	2.50
5	—	4.28	3.82
6	27.06	4.61	2.34
7	29.2	4.91	4.05
8	30.40	3.84	2.8
9	25.5	5.37	3.51
10	26.9	6.44	3.16
11	25.23	3.95	2.90
12	—	4.92	4.80

1 L and 1 S, 3 L, 4 S, 6 S, 8 S and 11 S seem to have a lower condensation coefficient. These differences may partly be due to the presence or absence of a few large or many smaller achromatic segments in the chromatic parts (cf. Figure 2), and partly due to various uncertainties with respect to the points to be employed for the measurements of these regions.

Two other possible causes for the variation in contraction factor should be mentioned. In the present study there may be a few wrong homologisations between pachytene and somatic metaphase chromosomes. Moreover, in measuring the individual somatic metaphase chromosomes, some wrong identifications within a group of very similar chromosomes may have occurred.

Discussion

It is clear from the present study that the structural differentiation into chromatic and achromatic parts is not a special characteristic of pachytene chromosomes alone, but also present in somatic metaphase chromosomes of tomato. The achromatic parts in somatic chromosomes seem to be stained with slightly higher intensity than those in pachytene chromosomes. Nevertheless, the differentiation into chromatic and achromatic parts is rather clear.

The presence of chromomeres in the chromatic parts of pachytene chromosomes has been observed by several previous workers (BROWN, 1949; BARTON, 1950; GOTTSCHALK, 1951, 1958; LIMA-DE-FARIA & SARVELLA, 1962). But the constant and characteristic structures that are of great value for the identification of pachytene chromosomes have not been systematically recorded previously. From the description of BARTON (1950), it is not possible to identify with certainty the chromosomes 5, 7, 8, 9 and 12. Chromosomes 8 and 9 have been described accurately by RICK & KHUSH (1964) and KHUSH et al. (1964). With the help of the structural details and identification markers recorded in Figure 2 for each chromosome, it is possible to identify all the chromosomes unambiguously. As already mentioned, these details fit all three varieties studied.

In spite of his extensive studies on the structure and length of pachytene chromosomes, GOTTSCHALK (1951) came to the conclusion that not all individual chromosomes can be identified when found isolated, but can be identified only in relation to one another. This was especially so for 7, 8, 9 and 10 of the present classification. Regardless of length and superficial resemblance between these chromosomes, they can be clearly distinguished from one another by employing the markers described here. Many chromosomes of the present description can be well compared with those of GOTTSCHALK (1951). Thus, chromo-

somes 1, 3, 7, 9, 10, 11 and 12 of GOTTSCHALK seem to be identical with 2, 8, 3, 9, 4, 6 and 1 of the present numbering, while others are at a variance. From the comparison of his photomicrographs, it seems that at least one of his chromosomes 6 in Figures 4 and 11 is misinterpreted, since they clearly correspond to 4 and 3 of the present classification. Many of the markers recorded in the present study are recognisable in the photomicrographs of Gottschalk.

The structural details and the so called pachytene gradients recorded by LIMA-DE-FARIA & SARVELLA (1962) are not at all comparable with the present observations. Except the nucleolar bivalent, on the basis of their drawings none of the other chromosomes can be identified with the present description of chromosomes. These great differences are certainly not due to varietal differences, since the present observation is based on the study of 3 varieties. Moreover, many of the structural details described here agree very well with those of BARTON (1950) and GOTTSCHALK (1951).

BROWN (1949) studied the length and ratio of the arms of the nucleolar chromosome (chromosome 2) of tomato, during different stages of meiosis and mitosis. Based on this study, he concluded that the highly asymmetric nucleolar chromosome of pachytene becomes "nearly symmetric", when it is contracted at somatic metaphase. This striking change in the arm ratio was explained as follows: The nucleolar chromosome, at pachytene, is composed of a short chromatic arm and a long arm containing a short, proximal, chromatic part and a rather long achromatic part, while the two chromatic parts on either side of the centromere are of about equal length; in somatic cells the achromatic part of the long arm of the pachytene chromosome is extremely contracted by the time the division cycle reaches metaphase, and it adds, according to BROWN, practically nothing to the length of the long arm and, therefore, the chromatic parts alone form the symmetric somatic metaphase chromosome.

It may be observed in Figure 2, that, although the achromatic part of 2 L (pachytene) is extremely contracted at somatic metaphase, a large "achromatic part" is still visible on the long arm of the satellite chromosome (Figure 3a and b) at somatic metaphase. This rather large achromatic part on the long arm was, as it seems, not taken into account by BROWN (1949), while considering the ratio of the two arms of the satellite chromosome. When the achromatic part is taken into

account, the satellite chromosome is clearly asymmetric also at somatic metaphase. Therefore, it is only a change from a highly asymmetric condition at pachytene to a less asymmetric condition at somatic metaphase. The figures and text of BROWN (1949), however leave some doubts with respect to his analysis of the satellite chromosome in somatic metaphase.

In all asymmetric pachytene chromosomes with asymmetric chromatic parts, the longer achromatic part is always the continuation of the longer chromatic part, but never of the shorter one. In chromosomes 3, 4, 9 and 10, which have asymmetric chromatic parts at pachytene, when neglecting the achromatic parts like BROWN (1949) seems to have done, the chromatic parts by themselves would form asymmetric chromosomes at somatic metaphase. In addition they have short achromatic parts on the long arms. The asymmetric chromosomes 1, 2, 6, 7 and 8 with about symmetric chromatic parts at pachytene, are asymmetric also at somatic metaphase, because of the presence of the achromatic segment in one of the arms. These achromatic segments obviously represent the long achromatic parts observed at pachytene, since, after a reduction in length by a factor of nearly 30, the short achromatic segments totally disappear on the short arms, whereas the long achromatic segments remain visible on the long arms. Therefore, even after a high degree of condensation of the achromatic parts, all asymmetric chromosomes remain asymmetric. Of the three symmetric pachytene chromosomes, only chromosome 11 has a rather long achromatic part associated with the shorter chromatic part. This inverse relationship is evident at somatic metaphase also.

Unlike the achromatic parts, the chromatic parts are, on the average, contracted only by a factor of five at somatic metaphase as compared to pachytene. These contraction factors and the difference between them are remarkably similar to the contraction coefficients of 25 and 5, reported for eu- and heterochromatin in *Oenothera* (JAPHA, 1939). In animal species five different contraction coefficients have been observed (SMITH, 1952).

It has been advocated that the chromosomal differentiation of tomato is similar to that of *Agapanthus*, *Oenothera*, *Pellia* and *Sphaerocarpus* (BROWN, 1949). In *Agapanthus* and *Oenothera*, it is reported that the chromosomal differentiation is a special characteristic of

meiotic chromosomes, not of mitotic chromosomes (DARLINGTON, 1937). This was explained as only due to precocious condensation of proximal parts during meiosis. Since in tomato the differentiation is clearly observed at somatic metaphase, when the chromosomes are in a state of maximum contraction, the differentiation may not merely be a consequence of precocious spiralisation, but probably originates from the differential organisation into chromatic and achromatic parts in tomato chromosomes.

The questions and problems regarding eu- and heterochromatin will be discussed in a later publication.

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