CHIASMA DISTRIBUTION AND SEGREGATION OF MARKERS IN CHROMOSOME 2 OF THE TETRAPLOID TOMATO¹

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THE relation of chromosome behaviour at meiosis to genetic segregation ratios in tetraploids is well established (LITTLE 1945, 1958). In the tetraploid tomato, chromosome 2 is readily identified at meiosis and, owing to the presence of heteromorphic satellites, the mode of pairing of the four homologues can be analyzed. In addition, chromosome 2 has a number of genetic markers which are suited to the investigation of tetraploid segregation.

In the following account the segregation ratios of several linked markers in 40,000 seedlings from selfed duplex tetraploids are presented. The behaviour of chromosome 2 at meiosis is analyzed and, based on the frequency of occurrence of two bivalents and quadrivalents, genetic segregation ratios are predicted.

MATERIALS AND METHODS

Tetraploid plants were obtained from seeds treated with colchicine (SWAMINATHAN 1950; BOHN 1947) and from callous tissue (WINKLER 1916). The original diploids were heterozygous for a number of markers and the resulting tetraploids were duplex (*AAaa*) for the markers. Diploids with heteromorphic satellites gave tetraploids with two No. 2 homologues which had long satellites, and two which had short satellites.

An incidence of 30 percent (37 tetraploids from 117 seeds) was obtained by using a colchicine concentration of .125 percent in a 1 percent agar solution, and by growing the seeds at room temperature for a period of 20 days. To prevent molding, the seeds were surface-sterilized in a solution of one part commercial bleaching solution and one part water for 5 min and subsequently washed in tapwater for ½ hour. Washing was done in vials capped with Fiberglas screen. After the colchicine treatment the stunted seedlings were kept on moist filterpaper for 10 days and then transplanted to soil. Many seedlings failed to produce roots and died. The surviving plants were grown to maturity at which time buds were collected from each plant and examined at meiosis to determine the chromosome number.

Some 50 different tetraploids were produced. The mature plants as well as cuttings from the most favourable plants were planted in the field, and fruits were collected in the fall. The seeds were planted and the seedlings segregated in the greenhouse during the winter.

To minimize the effect of genetic variability between plants, clones were vegetatively produced from single plants. That is, one diploid seed of genotype $\frac{dv + aw +}{+ d + uv}$ gave tetraploid plant M-10-1 of which ten cuttings were made and grown to maturity. The 10,000 seedlings from M-10-1 plants can be considered to have come from one parent plant. Table 1 lists the main tetraploid plants used for this research with their diploid origin.

¹ Based on a thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology at the University of Toronto (1963).

TABLE 1

Duplex tetraploid plants and their diploid origin

Diploid origin	Diploid genotype	Clone number
One seed	$\frac{d\ wv}{++} \frac{c}{+}$	M-4-3
One seed	$\frac{dv + aw}{+} \frac{+}{d + wv}$	M-10-1
One diploid plant (callous tissue)	$\frac{d\ m\ aw}{++} \frac{+}{+} \frac{c}{wv} \frac{a}{+}$	X6142-4n

The following markers were used: *a*, green stem (chromosome 11); *aw*, green stem (chromosome 2); *d*, dwarf, a compact, dark green plant (chromosome 2); *dv*, dwarf virescent, a spindly plant with yellowish new leaves (chromosome 2); *c*, potato leaf (chromosome 6); *Me*, mouse ear, leaves several times pinnately divided (chromosome 2); *m*, mottled, leaves have yellow spots (chromosome 2); *s*, compound inflorescence, many-branched inflorescence (chromosome 2); *wv*, white virescent, new leaves white, later becoming green (chromosome 2). The markers are listed and described by BARTON, BUTLER, JENKINS, RICK and YOUNG (1955). RICK, and BUTLER (1956), and BUTLER (1960), and *wv* has been reported by RICK (1960).

For the investigation of chromosome behaviour in the tetraploid, small buds were collected in capped vials with aceto-alcohol 1:3. A modified acetocarmine technique was used for staining (MOENS and BUTLER 1963). Prior to mordanting in 4 percent iron alum, the buds were hydrolyzed in hot N HCl (60°C) for 10 minutes. Squashes were found to be improved by the hydrolysis but considerable rinsing was necessary to eliminate the HCl. Final staining density was controlled by steaming and by the use of 45 percent acetic acid. Slides were made permanent with the dry ice technique.

RESULTS

The genetic segregation ratios are presented in Table 2 (a), (b), (c) and (d). The first three sections of this table list the segregation of seedlings from each of the three clones. The last section is a summary. From the table it is apparent that all markers of chromosome 2 give ratios in the order of one recessive (nulliplex, *aaaa*) plant to 30 normal plants. Due to the large numbers involved, the standard errors of the ratios are small (Table 2 d), and the monogenic ratios do not differ significantly from the average ratio of 1:30.3.

Where linked markers in coupling phase were present, the "parentals" and "recombinants" are reported in Table 2. Neither term is correctly applied, but they are used here for convenience. Tightly linked markers such as *d-m* (2 percent recombination in the diploid) give few "recombinants" whereas for *d-wv* (31 percent recombination in the diploid) many "recombinants" are found.

Cytological studies of chromosomes at meiotic prophase revealed that multi-valent formation of chromosome 2 was of a specialized type. Chromosome 2 has only one long euchromatic arm, the short arm is heterochromatic and carries the nucleolus organizer and the satellite (Figure 1). Chiasma formation and crossing over is known to occur only in the long arm (BROWN 1949; BARTON 1951; MOENS

TABLE 2

Segregation ratios of the progeny of selfed duplex tetraploids

(a) <i>Clone M-4-3</i>					
Marker	Recessives	Normals	Ratio		
<i>d</i>	493	14,747	1 : 29.9		
<i>wv</i>	493	14,747	1 : 29.9		
<i>c</i>	431	14,809	1 : 34.4		
<i>Linked markers (Coupling phase):</i>					
<i>d-wv</i>		+ + 14,436	x + 311	+ y 311	x y 182
(b) <i>Clone M-10-1</i>					
Marker	Recessives	Normals	Ratio		
<i>dv</i>	300	9,189	1 : 30.6		
<i>aw</i>	309	9,180	1 : 29.7		
<i>d</i>	275	9,214	1 : 33.5		
<i>wv</i>	296	9,193	1 : 31.1		
<i>Linked markers (Coupling phase):</i>					
<i>dv-aw</i>		+ + 9,094	x + 86	+ y 95	x y 214
<i>d-wv</i>		9,024	169	190	106
(c) <i>Clone X6142-4n</i>					
Marker	Recessives	Normals	Ratio		
<i>a + aw</i>	731	12,976	1 : 17.8		
<i>c</i>	478	13,229	1 : 27.7		
<i>wv</i>	427	13,280	1 : 31.1		
<i>d</i>	468	13,239	1 : 28.3		
<i>m</i>	444	13,263	1 : 29.9		
<i>Linked markers (Coupling phase):</i>					
<i>d-m</i>		+ + 13,217	x + 46	+ y 46	x y 422
(d) <i>Totals for chromosome 2 markers</i>					
Marker	Recessives	Normals	Ratio	Standard error of the ratio	
<i>dv</i>	300	9,189	1 : 30.6	± 1.8	
<i>m</i>	444	13,263	1 : 29.9	± 1.5	
<i>d</i>	1,236	37,200	1 : 30.1	± .9	
<i>aw</i>	309	9,180	1 : 29.8	± 1.8	
<i>wv</i>	1,216	37,220	1 : 30.6	± .9	
Total:	3,505	106,052	1 : 30.3	± .5	

and BUTLER 1963). It was found that in the tetraploid the four homologues form bivalents and multivalents and that the homologues participating in a multivalent have chiasmata and partner exchanges in the long arm only. The types of rings-of-four and chains-of-four which can theoretically be distinguished, in the presence of heteromorphic satellites, are outlined in Figure 2. A few photographic examples and the interpretative drawings are given in Figure 3.

A total of 386 meiotic prometaphase cells were examined to obtain an estimate of the frequency of occurrence of the different types of associations of chromosome 2 homologues (Table 3).

FIGURE 1.—Diagram of chromosome 2 at pachytene and the associated genetic map in the diploid tomato. Crossing over occurs only in the long euchromatic arm which has a length of about 30 microns at pachytene. Seven of the 20 known markers are given; the markers *s* and *dv* are terminal.

TABLE 3
Chromosome association in the tetraploid tomato

		Number of cells observed
Bivalents		
with unlike satellites		175
with like satellites		92
Quadrivalents		
rings, A		14
B		14
C		20
chains 2 × 2, A		11
B		2
C		7
D		3
chains 1-2-1, A		12
B		9
C		13
D		8
Trivalents		6
Total		386
Bivalents		: 69.2%
Quadrivalents		: 29.2%
Trivalents		: 1.6%

FIGURE 2.—Types of chromosome 2 quadrivalents in the tetraploid tomato. Due to the presence of heteromorphic satellites (dark areas) next to the centromere, different forms can be distinguished. (a) Ring quadrivalents with two proximal and two distal crossovers and an exchange of partners between the crossovers. (b) Chain quadrivalents having two proximal and one distal chiasmata. (c) Chain quadrivalents with one proximal and two distal chiasmata. (d) Drawings from photomicrographs illustrating the two possible forms of bivalents and different types of rings-of-four.

FIGURE 3.—Photomicrographs and drawings of chromosome 2 bivalents and chains-of-four in a tetraploid tomato which has two homologues with long satellites and two homologues with short satellites. (a) The four homologues have paired to form two bivalents. One bivalent has two short satellites, the other two long satellites. (b) A chain-of-four, “two-by-two” type A (Figure 2b). (c) A chain-of-four, “one-two-one,” type B (Figure 2c). (d) A chain-of-four, “one-two-one,” type D (Figure 2c).

DISCUSSION

The gametic series for any factor in duplex auto-tetraploids will be dependent on: (1) the nature of chromosome pairing at early prophase; (2) the mean and variance of the frequency of crossing over; and (3) the disjunction of the resulting multivalent (MATHER 1935, 1936; LITTLE 1945).

In a selfed, heterozygous, diploid plant the theoretical segregation into dominant and recessive is 3:1, irrespective of the location of the factor in relation to the centromere. In a duplex tetraploid, the segregation ratios vary with the distance from the centromere. The variations in ratios and the underlying mechanisms have been studied by MULLER (1914); HALDANE (1930); and MATHER (1936).

(1) *Random chromosome segregation (reductional segregation)*: In this type of segregation, proposed by MULLER, it is assumed that pairing between any two of the four homologous chromosomes took place at random. Thus the duplex $AAaa$ will give gametes in a ratio of 1 AA :4 Aa :1 aa , i.e., one sixth of the gametes is double recessive. On this basis it was concluded that 1/36th of the zygotes will be nulliplex ($aaaa$).

MULLER's ratio holds where the four homologous chromosomes form two bivalents, and the ratio therefore partially applies to chromosome 2 markers in the tomato where 70 percent of the pollen mother cells have bivalents. MULLER's ratio also holds for markers close to the centromere in the case of multivalents. However, random chromosome segregation fails to account for the observed fact that recessive zygotes usually occur in excess of 1:35.

(2) *Random chromatid segregation*: This type of segregation was proposed by HALDANE in 1930. For autotetraploids it was shown that, if one assumed random assortment of chromatids rather than of chromosomes, a duplex hybrid would give a ratio of 9:187 (or approximately 1:21) on selfing. This ratio is arrived at by taking all combinations of two alleles from *AAAAaaaa*. The following gametic series is obtained: *3AA:8 Aa:3 aa*, that is 3/14 of all gametes are recessive. Of the resulting zygotes 9/196th are recessive, giving a ratio of 9:187. HALDANE's approach fits the observed ratios better than MULLER's in that it can account for the exceptional recessives obtained from *AAAA* × *AAAA* and *AAAA* × *aaaa* in *Datura* (BLAKESLEE, BELLING and FARNHAM 1923). According to MULLER, the plant *AAAA* cannot produce recessive gametes whereas, according to HALDANE, such a plant will produce one *aa* gamete in 28 gametes.

MATHER (1935) examined HALDANE's ratios, and states, "These ratios imply (a) random pairing and exchange of partner among the chromosomes at zygotene; (b) infinite crossing over between the gene and the attachment; and (c) random disjunction." It should be added that the ratios also assume that multivalents are formed all the time.

The implicit assumption that infinite crossing over occurs between the gene and the centromere is unrealistic in the case of chromosome 2 of the tomato, since diploid segregation indicates a maximum of two crossovers for the long arm and observations at diplotene in the diploid and the tetraploid indicate a maximum of two chiasmata for the long arm.

(3) *Equational separation*: MATHER (1935) considers the segregation of a marker in a multivalent which has one chiasma between the locus of the gene and the spindle attachment. For random chromosome pairing and random disjunction of the quadrivalent into two and two, the gametic series for the duplex plant will be: *2 AA:5 Aa:2 aa*, or two-ninths *aa* gametes, and the frequency of recessive zygotes will be $(2/9)^2 = 4/81$, a ratio of about 1:19.

To characterize an observed ratio in terms of cytological behaviour, MATHER (1936) introduced the index α which was called "the simple index of separation." The index α is the product of two factors, a and e . a = the frequency with which adjacent chromosomes of a quadrivalent go to the same pole. The factor a has a random value of 1/3. e = the amount of equational separation of chromatids. Since the separation at the first division is always reductional at the centromere, equational separation at a given locus depends on the chiasma frequency between the locus and the centromere. The upper limit of α should be one third where there is one crossover between the gene and the centromere and where random disjunction of quadrivalents takes place. The frequency of recessive gametes in the duplex plant now becomes $f(aa) = (1 + \alpha)/6$. MATHER (1936) reserves the index alpha for the gametic series from simplex and triplex plants but FISHER and MATHER (1943) argue that one index will suffice for all types of segregation.

From the cytological evidence obtained in tomato tetraploids, an expected frequency of recessive zygotes can be calculated. Let p be the average frequency with which a set of four homologues form a quadrivalent.

$$f(aa) = (1 - p)/6 + p(1 + \alpha)/6$$

The first term represents the yield of recessive gametes from cells with bivalents, 70 percent in this case, and the second term gives the yield of recessive gametes from cells with multivalents. If α is taken at 1/3, the expected frequency of recessive zygotes is

$$f(aaaa) = \left(\frac{1 + P\alpha}{6}\right)^2 = \left(\frac{1 + .3 \times 1/3}{6}\right)^2 = \frac{1}{29.8}$$

or roughly one recessive plant per 30 progeny. The assumptions are that there is

random pairing of the homologues, random disjunction, and, in the case of multivalents, that there is one crossover between the centromere and the marker.

The first assumption is appropriate as far as the bivalents are concerned. In a tetraploid which has two chromosomes with short satellites, and two with long satellites, random pairing results in two thirds of the pollen mother cells having bivalents with unlike satellites, and one third of the cells having bivalents with like satellites (Figure 2d, "Bivalents," and Figure 3a). It was observed that in 267 cells which had chromosome 2 bivalents, 175 cells had bivalents with unlike satellites and 92 with like satellites. Comparison with the random values gives a chi-square value of .15 with a P value of .7. The large number of classes of multivalents and the relatively small number of observations per class makes it difficult to test multivalents for random pairing. However, the fact that the tetraploids were of recent origin and that the chromosomes associate randomly in bivalents, allows random pairing to be accepted as a valid assumption.

The validity of the second assumption, random disjunction, can not be tested directly. From Figures 2 and 3 it can be seen that the centromeres near the proximal chiasma are held close together. If it is argued that such centromeres repel each other and that they will go to opposite anaphase poles (DARLINGTON 1937) then markers of chromosome 2 would segregate according to MULLER's ratio of 1:35. The present results and SANSOME's (1933) results contradict such a hypothesis. If, on the other hand, the centromeres which are held together by the proximal chiasma tend to go to the same pole, a high yield of recessive gametes from multivalents results ($f(aa) = 1/3$). The frequency of recessive zygotes would be $f(aaaa) = (7 \times 1/6 + .3 \times 1/3)^2 = 1/21.3$, which is in contradiction with the present results.

The third assumption involves the frequency of crossing over between the centromere and the markers. The genetic distance from the centromere to the markers was determined in the diploid by MOENS and BUTLER 1963 (Figure 1). The recombination percentage was determined directly. Due to the absence of crossing over in the short arm, the satellite is a cytological marker for the centromere, and by using diploid plants heterozygous for chromosome 2 genes and having heteromorphic satellites it was found that the centromere is at the *s* end of the genetic map, 31 units from *s*.

The distribution of crossovers in the diploid bears on the tetraploid segregation. Only the multivalents can produce recessive gametes in excess of one in six, and in the case of chromosome 2, crossovers and partner exchanges are restricted to the long arm (Figure 2). Each of the long arms in the rings-of-four must have two crossovers and a partner exchange between the crossovers. The chances of having the first crossover proximal to the first marker, *wv* here, are in excess (about 5×) of having both crossovers distal to *wv*. The occurrence of a proximal crossover is especially favoured in the rings-of-four by the fact that the two crossovers are separated by an exchange of partners. Since, in the diploid, the long arm appears to support a maximum of two crossovers, it is likely that the additional exchange of partners in the tetraploid interferes with random distribution

of crossovers, allowing crossovers only in proximal and distal positions. Furthermore, observations show that multivalent configurations at diplotene have closely appressed heterochromatic areas suggesting a chiasma near the centromere (Figures 2c, 3). The interpretation of diplotene configurations, however, depends on the correlation that exists between chiasma and crossing over.

If homologues pair randomly to form rings-of-four, then types A, B and C occur with equal frequency. The gametic frequency for a marker, *a*, distal to the proximal chiasma, will be $f(aa) = 1/3 (1/4 + 1/4 + 1/6) = 2/9$, in conformity with MATHER's values. A terminal marker, *b*, separated by two chiasmata from the centromere, will give $f(bb) = 1/3 (1/4 + 5/24 + 5/24) = 2/9$. The chains of "two-by two" have the same gametic frequencies as the rings-of-four and the chains of "one-by-two-by-one" have very similar values $f(aa) = 7/36$; $f(bb) = 2/9$.

From the above values it appears that where there is random pairing and random segregation of the No. 2 homologues, the overall frequency of recessive gametes is 2/9 for the duplex tomato. It follows that the expected frequency of recessive zygotes is $f(aaaa) = [(.7 \times 1/6) + (.3 \times 2/9)]^2 = 1/29.8$. This frequency is based on 380 observations and has an error associated with it. The expected value can be expressed as one recessive zygote in $29.8 \pm .4$ plants.

The expected value may also be derived from the frequency with which each type of quadrivalent actually did occur in the observed sample of 113 quadrivalents. Each type has its own expected number of recessive gametes associated with it. The frequency of occurrence and the genetic frequency for each type of multivalent were combined and an expected frequency of one recessive zygote in 31.25 plants was obtained for a marker separated by one crossover from the centromere. The same calculations for a marker separated from the centromere by two crossovers gave a frequency of 1/29.90.

The hypothetical values are in close agreement with the observed frequency of 1/31.3. It is concluded that the tetraploid segregation ratios for markers on chromosome 2 do not show differences and that the absence of differences is due to the asymmetric structure of chromosome 2 which causes the formation of specialized multivalents having proximal crossovers close to the centromere.

From the above study it appears that caution is necessary in the use of tetraploid segregation ratios to determine the position of a marker relative to the centromere. The fact that ratios of 1 to 35 are obtained does not necessarily imply a location of the marker close to the centromere, but may indicate the absence of multivalents for the chromosome. The structure of the chromosome determines the frequency and the type of multivalents formed and it is, among others, necessary to know the frequency with which the four homologues associate to form a multivalent before a conclusion can be reached on the location of the markers. The average number of multivalents per cell cannot be used as an estimate for frequency of multivalents of one specific chromosome since some chromosomes may form multivalents more frequently than others. There appears to be no virtue in testing tetraploid ratios against the standard 1 to 35 or 1 to 20

ratios, since factors other than the distance to the centromere are effective in altering tetraploid ratios.

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SUMMARY

The segregation of 40,000 progeny from selfed duplex tetraploid tomatoes (*AAaa*) is presented. The average segregation ratio of five markers on chromosome 2 is found to be one recessive plant (*aaaa*) to 30.3 normal plants. The ratios for the individual markers do not differ significantly from the average. The diploid genetic map of chromosome 2 gives *wv* at 38 units and *dv* 74 units from the centromere. It appears that the various diploid distances are not reflected in the tetraploid ratios.

The structure and behaviour of chromosome 2 at meiosis is described. The satellite is a cytological marker for the centromere and with the aid of heteromorphic satellites it was possible to ascertain random pairing of the four homologues at prophase, and to analyse multivalent structures. It is shown that the asymmetric structure of chromosome 2 is responsible for the formation of an unusual type of quadrivalent. In the ring-of-four the long arm of each homologue has a proximal crossover close to the centromere, an intermediate exchange of partners and a distal crossover. In 386 pollen mother cells, 267 cells (69 percent) had two bivalents, 113 cells (29 percent) had quadrivalents, and six cells (2 percent) had a trivalent for chromosome 2.

Based on the cytological behaviour of chromosome 2, expected genetic segregation values are calculated. The hypothetical values correspond to the observed values. It is argued that genetic segregation ratios are not necessarily a function of the distance from the marker to the centromere, but also depend on the structure and behaviour at meiosis of the chromosome in question.

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