TOMATO SECONDARY TRISOMICS : ORIGIN, IDENTIFICATION, MORPHOLOGY, AND USE IN CYTOGENETIC ANALYSIS OF THE GENOME

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1. INTRODUCTION

SECONDARY trisomics, *i.e.* individuals having an isochromosome in addition to the normal somatic number, have been least investigated among the known kinds of trisomics. The lack of any cytogenetic technique for the direct experimental production of secondary trisomics probably accounts for the rarity of studies on this subject. Definitely proven cases of secondary trisomy have been reported only in *Datura*, maize, tomato, and wheat. In *Datura stramonium*, 14 of the 24 possible secondary trisomics were established (Blakeslee and Avery, 1938). Only one secondary trisomic of maize (Rhoades, 1933, 1940) and one of wheat (Sears, 1954) and two of tomato (Sen, 1952; Moens, 1965) have been reported to date.

The identification of secondary trisomics was confirmed in *Datura stramonium* by extensive breeding tests and in maize and tomato by the highly diagnostic pachytene analysis. Despite their utility for finding the arm location of the markers, thus ascertaining the position of the centromeres and the orientation of linkage maps, secondary trisomics have not been employed heretofore for such analysis in any species.

In recent work, we have isolated nine secondary trisomics of tomato including one reported by Sen (1952). Five of these were employed in determining the arm location of marker genes. Knowledge of the tomato genome as well as understanding of secondary trisomy in general has been considerably enhanced by these investigations on morphology, genetics, cytology and transmission rates.

2. ORIGIN

Sen (1952) working with tomato and Sears (1954) with wheat were the first to isolate secondary trisomics amongst the progenies of plants in which a normal chromosome of the complement had been replaced by an isochromosome for one of its arms. Such individuals which are haploid for one arm and triploid for the other arm of the same chromosome were called mono-isosomes by Sears (1954) and haplo-triplo-disomis (HTD's) by Khush and Rick (1967*b*). We were able to establish seven HTD's in tomato, from five of which large progenies were raised, three yielding secondary trisomics. A procedure for obtaining haplo-triplo-disomics, thereby providing a source of secondary trisomics, was also outlined by Khush and Rick (1967*b*). Three

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secondary trisomics—2n+8L·8L, 2n+9L·9L and 2n+10L·10L—were thus produced experimentally.

Three secondary trisomics were isolated from the progenies of double iso-compensating trisomics. In such trisomics, one normal chromosome of the complement is missing, but it is compensated by two isochromosomes, one for each of the two arms. Two compensating trisomics of this kind—2n − 7S·7L + 7S·7S + 7L·7L and 2n − 3S·3L + 3S·3S + 3L·3L—have been obtained in tomato (Khush and Rick, 1967a). In the same publication we predicted that 2n − 3S·3L + 3S·3S + 3L·3L should yield 2n + 3S·3S in its progeny but not 2n + 3L·3L. The unbalance caused by the extra 3L·3L is not tolerated by the gametophytic generation (Khush and Rick, 1967b). Similarly, the appearance of 2n + 7S·7S and 2n + 7L·7L in the progeny of 2n − 7S·7L + 7S·7S + 7L·7L was also predicted. The data on the backcross progenies of these two trisomics are presented in table 1. As predicted, both

TABLE 1

Backcross progenies of double-iso compensating trisomics

						Progeny					
						Parental 2n+1		Secondary trisomic for short arm		Secondary trisomic for long arm	
Trisomic	Total	2n		2n+1		for short arm		for long arm			Other
		No.	%	No.	%	No.	%	No.	%	
2n − 3S · 3L+3S · 3S+3L · 3L	283	195	68·8	68	24·0	16	5·6	0	0·0	4*
2n − 7S · 7L+7S · 7S+7L · 7L	185	116	62·7	48	25·9	20	10·8	1	0·54	0

* One was triplo-11 and 3 were HTD (see text).

of the compensating trisomics yielded secondary trisomics for short arms among their progenies at a relatively high frequency (table 1, columns 7 and 8); no secondary trisomic for 3L appeared, but one individual of 2n + 7L· L7 constitution was produced. On the basis of cytological observations, a much higher frequency of 2n + 7L·7L individuals would be expected, but the poor viability of the n + 7L·7L gametophytes is probably responsible for low yield obtained. Three plants in the progeny of 2n − 3S·3L + 3S·3S + 3L·3L were HTD's (2n − 3S·3L + 3L·3L). The argument that n − 3S·3L + 3L·3L gametes are viable might seem to be favoured by the appearance of these HTD's but not by the failure of the earlier tested 2n − 3S·3L + 3L·3L to transmit its karyotype to a large progeny (Khush and Rick, 1967b). Therefore, these three HTD's must have originated by somatic loss of the 3S·3S chromosome during early ontogeny from offspring that initially were 2n − 3S·3L + 3S·3S + 3L·3L. This explanation seems plausible also in view of the fact that the telocentric ·3S from which the 3S·3S isochromosome originated was also somatically unstable (Khush and Rick, 1967a).

Of the other three secondary trisomics, 2n + 9S·9S and 2n + 6L·6L originated spontaneously in the progenies of haplo-6S·7S and haplo-4S·10S, respectively (Khush and Rick, 1966a). The remaining one—2n + 12L·12L —is the only secondary trisomic which originated on three independent occasions. It appeared spontaneously, first in the progeny of a plant deficient

for 4S (Khush and Rick, 1967c) and second in the progeny of haplo 3S-triplo 3L. Finally, it was isolated among the progeny of haplo-10S·12S. In the latter family, the isochromosome 12L·12L probably resulted from the fracture of the tertiary chromosome 10L·12L in the centromere region, and its inclusion in a gamete carrying n chromosomes led to the production of said trisomic.

Thus, in summary, our set of nine secondary trisomics appeared among the progenies of haplo-triplo-disomics, double iso-compensating trisomics, tertiary monosomics and segmental deficiencies. The mechanism that leads to the production of secondary trisomics seems clear. First, an isochromosome is produced by breakage of the centromere and union of identical arms. Second, the isochromosome must be included in a gametophyte in addition to the entire haploid chromosome complement, and such a gametophyte must be viable. The isochromosomes 2L·2L, 3L·3L and 6L·6L produce so much unbalance as extras that the gametophytes fail to function—a topic that is treated in detail in a subsequent section. Third, the n+isochromosome gamete must be fertilised by an n gamete and the resulting zygote must be viable.

Until recently, no way of producing isochromosomes experimentally was known (Avery, Satina and Rietsema, 1959, p. 101). We have shown, however, (Khush and Rick, 1967b) that isochromosomes can be produced by irradiating pollen. The hypothesis of Belling and Blakeslee (1924) that isochromosomes arise by crossing over between sister strands that lie side by side, but with the ends of one reversed, seems less probable.

3. Cytology

Familiarity with the gross morphology of primary trisomics is often helpful in identifying new trisomic types, but may be untrustworthy as the sole criterion according to the present research; conclusive evidence is provided by cytological examination. The secondary trisomic condition can be readily diagnosed by the presence of ring trivalents. Pachytene configurations (figs. 1-18) are then easily analysed to identify the isochromosome, which may be present as a univalent when paired internally (figs. 1, 4, 7, 8, 9, 12, 14 and 15). Chromomere-by-chromomere pairing is clearly evident and terminal centromeres can be seen in all of these figures. Frequently the isochromosome is paired with the two normal homologues to form a trivalent (figs. 2, 3, 5, 6, 10, 11, 13, 16, 17 and 18). When univalent, the isochromosome forms a small ringlet. Evidently, internally paired isochromosomes invariably form at least one chiasma. Cells with three univalents were extremely rare. The frequency of cells with trivalents was highly variable (table 2). In general, secondary trisomics for long arms formed trivalents more frequently than those for short arms, as expected on the basis of opportunity for pairing and chiasma formation.

The trivalents assumed various configurations. The eight different types observed and their frequencies in each of the trisomics are shown in table 2. These different configurations result from a variable number and position of chiasmata. The pachytene associations and the number and position of chiasmata that give rise to these configurations at diakinesis are shown diagramatically in fig. 20. It may be noted that the frequency of configurations of type g was lowest. In fact, we observed only two PMC's

TABLE 2

Chromosomal associations at diakinesis
(50 cells each)

Trisomic	3_I	II+I	Type a	Type b	Type c	Type d	Type e	Type f	Type g	Type h	% cells with		
											$11_{II}+3_I$	$12_{II}+1_I$	$11_{II}+1_{III}$
2n+3S·3S	0	28	8	5	3	3	3	0	0	0	0	56	44
2n+7S·7S	0	34	8	2	1	0	3	0	0	2	0	68	32
2n+9S·9S	2	28	3	6	4	4	5	0	0	1	4	56	40
2n+6L·6L	0	16	4	5	4	2	12	5	0	2	0	32	68
2n+8L·8L	0	21	5	6	3	2	9	2	0	2	0	42	58
2n+9L·9L	0	19	5	5	4	3	12	1	0	1	0	38	62
2n+10L·10L	0	20	8	5	3	3	8	0	0	3	0	40	60
2n+12L·12L	0	14	6	6	3	1	16	1	2	1	0	28	72

Chromosomal associations*
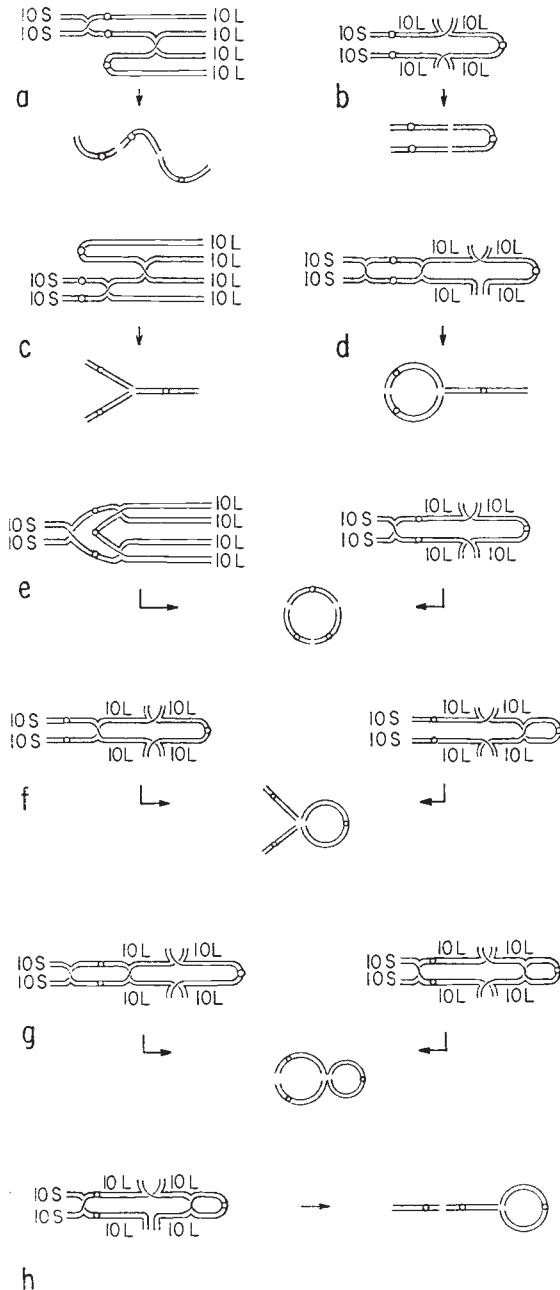
* See fig. 20 for types of chromosomal association.

FIG. 20.—Patterns of chromosomal association and chiasma formation at pachytene in the trivalent of the secondary trisomic 2n+10L · 10L. Arrows point to the respective configurations anticipated at diakinesis.

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with trivalents of this type, both in $2n + 12L \cdot 12L$. The fact that such a trivalent requires the highest number of chiasmata probably explains its rarity. The trivalents of types f and h were also infrequent, ring and chain trivalents (types a to e) being more common. Rings were more frequent in the secondaries for long arms than in those for short arms.

The later stages of meiosis were normal. When present as a univalent the isochromosome passed to one of the poles at anaphase I, laggards having appeared rarely. The orientation of the trivalent at metaphase I and the subsequent segregation of the three chromosomes at anaphase I deserve special mention. In the secondary trisomics for such long arms as 6L, 8L, 9L and 10L, the orientation of the trivalent frequently favoured the separation of the isochromosome to one pole and the two normal chromosomes to the other, probably for mechanical reasons. Consisting of two long arms, the isochromosome is much longer than the entire normal chromosome and tends to orient itself toward one pole, the two normal chromosomes toward the other. An example may be seen in the ring trivalent of $2n + 10L \cdot 10L$ shown in fig. 19. In trivalents of the secondary trisomics for short arms, such as 3S, 7S and 9S, the reverse is true. The isochromosome in these trisomics is shorter than the two normal chromosomes, and the orientation of the trivalents tends to result in the two normal chromosomes passing to different poles and the isochromosome segregating at random. Since the centromere of chromosome 12 is median, the three members of the trivalent in $2n + 12L \cdot 12L$ are identical in size, the isochromosome could not be distinguished, consequently the orientation and segregation of the members of the trivalent could not be determined. Because of their similar size, the orientation and segregation was probably random.

This variable behaviour of the trivalents of different secondary trisomics at metaphase-anaphase I has an obvious bearing on the gametic output. Four kinds of gametes can be produced by any secondary trisomic by two types of segregations of three chromosomes of the trivalent. For example, from a trivalent $10S \cdot 10L - 10 \cdot L10L - 10L \cdot 10S$ of $2n + 10L \cdot 10L$, the $10S \cdot 10L$ and $10L \cdot 10L$ chromosomes may go to one pole and $10S \cdot 10L$ to the other. This type of segregation should produce n and $n + 10L \cdot 10L$ gametes. The segregation in which the $10L \cdot 10L$ chromosome goes to one pole and the two $10S \cdot 10L$ chromosomes to the other should produce $n - 10S \cdot 10L + 10L \cdot 10L$ and $n + 10S \cdot 10L$ gametes. The gametes of the former type ($n - 10S \cdot 10L + 10L \cdot 10L$) must certainly abort due to the deficiency of one arm (10S). Had such gametes been viable they would have produced haplo-triplo-disomics. As already shown by Khush and Rick (1967b), such gametes never function. Therefore, the three types of functional gametes produced by a secondary trisomic such as $2n + 10L \cdot 10L$ are n, $n + 10L \cdot 10L$ and $n + 10S \cdot 10L$, producing diploids, secondary trisomics and the related primary trisomics, respectively, upon backcrossing.

The appearance of related primary trisomics in the progenies of the secondary trisomic is one of the very important features of these trisomics— a subject that will be treated in later sections. It should be emphasised here, however, that the frequency of $n + 1$ gametes in which the extra chromosome is primary would depend upon the frequency of trivalent formation and the type of segregation it undergoes at anaphase I. The extra isochromosomes for the short arms form trivalents less frequently, which, when formed, mainly segregate to produce n and $n + 1$ gametes in which the extra chromosome is

the isochromosome. With the secondary trisomics for long arms, the cytological conditions for production of $n+1$ gametes with a primary extra chromosome are more favourable.

4. Morphology

The secondary trisomics of tomato differ to varying degrees from diploid, in vigour and growth habit. The secondaries for the long arms, 6L, 7L, 8Ls 9L and 10L, have much slower growth rates at all stages of growth than those for the short arms. Moreover, the secondaries for the long arms are sometimes difficult to distinguish from the corresponding primaries. Most of the characters of the primaries tend to be exaggerated in the secondaries, all of the latter being distinguishable from the normal sibs at the cotyledonary or first true leaf stages. On the other hand, the seedling vigour of the secondaries for the short arms such as 3S, 7S and 9S is scarcely reduced and the morphology of their seedlings is not modified to any appreciable extent. Thus, as seedlings, $2n+3S \cdot 3S$ and $2n+9S \cdot 9S$ cannot be separated from the diploids and $2n+7S \cdot 7S$ only with difficulty. Under field conditions, $2n+9S \cdot 9S$ is rather weak and strikingly different in growth habit, but $2n+3S \cdot 3S$ and $2n+7S \cdot 7S$ are almost as vigorous as diploids. The remaining secondary, $2n+12L \cdot 12L$, is quite distinct at all stages of growth, but it shows only about half of the characters of its primary.

The following morphological descriptions of the secondary trisomics are based on the observations of both greenhouse and field-grown plants. Observations on $2n+6L \cdot 6L$ and $2n+7L \cdot 7L$ were limited to greenhouse cultures of the single plant of each that have been obtained to date.

$2n+3S \cdot 3S$. Plant vigorous and fruitful. Resembles triplo-3 in being somewhat slender and in having flower buds which taper towards the pedicel, thus sharply demarcating the receptacle; pedicel sharply recurved, up to 180°, appearing like a hook; the inflorescences highly pubescent; terminal meristems resembling the *Hrt* mutant. The hairless appearance of triplo-3 must be controlled by 3L. In respect to hair density, the two arms thus seem to act in opposite directions.

$2n+6L \cdot 6L$. Plant very weak and unfruitful; distinguishing features of triplo-6 exaggerated; all the plant parts including internodes, leaf segments, inflorescences and flowers elongate. The highly elongated internodes of the seedling render it so different from diploid sibs, in fact, that they provoked cytological examination and identification. All parts of the inflorescence are extremely elongate; the characteristic leaf texture of triplo-6 magnified. Like triplo-6, this trisomic is somewhat fragile.

$2n+7S \cdot 7S$. Plant vigorous and fruitful; leaves curled and serrate like those of triplo-7. But in all of the other distinguishing characters this trisomic is the antithesis of triplo-7. Triplo-7, for example, has compact and bushy growth habit, thickened stems, compact internodes and inflorescences, and disc-like fruit, whereas $2n+7S \cdot 7S$ has open growth habit, slender stems and other plant parts, elongated internodes, inflorescences and flowers, and somewhat elongated fruits.

$2n+7L \cdot 7L$. Plant very slow and weak; most of the characters of triplo-7 exaggerated: for example, stems very stiff and heavy with small internodes; leaves extremely curled with deeper but fewer serrations; tendency

towards blindness extremely pronounced, every branch losing its growing point after producing 2-3 leaves. Due to the latter defect, the plant produced flower buds only after 10 months of growth and none of its flowers have set fruit yet.

$2n + 8L \cdot 8L$. Plant very weak and slow growing, half the size of triplo-8; growth habit open and erect; all other characters of triplo-8 magnified: cotyledons much thicker, broader and leathery; internodes much shorter; leaves entirely convex, greyish, deep veined and thickened with very little serration and with fewer divisions; flower parts, including the pedicels, reduced in size; flower partially cleistogamous, stigma exserted; and fruits flattened and white. Moderately fruitful under greenhouse conditions.

$2n + 9S \cdot 9S$. Plant very vigorous in the seedling stage but later growth much retarded; highly unfruitful in the greenhouse and under field conditions. The trisomic shows no resemblance to triplo-9 except for a tendency for blindness. Leaves simple with secondary leaflets less serrate and more rounded; inflorescence, in contrast with the compound structure of triplo-9, simple with very few abortive flowers; flowers with thickened receptacle and long and dialytic anthers; flowers and fruits larger than those of triplo-9.

$2n + 9L \cdot 9L$. Plant very diminutive and slow growing, one-third the size of triplo-9. All the characters of triplo-9 are highly exaggerated in this trisomic: growth habit erect; leaves folded, plicate; inflorescence compound; flowers and fruits smaller; stem colour yellow-green; internodes reduced in size and number; plant somewhat brittle; moderately fruitful under greenhouse conditions.

$2n + 10L \cdot 10L$. Plant slow growing and about half the size of triplo-10. In common with all the secondaries for long arms, all the characters of the corresponding primary (triplo-10) are exaggerated in this trisomic: stem thicker; growth habit stocky and bushy; internodes very short; inflorescence compact; flower parts reduced in size; stigma exserted; undulations of leaf margins extremely pronounced; terminal leaf segment twisted. Unlike triplo-10, flower buds have intense anthocyanin, even in summer, and calyx is longer than corolla.

$2n + 12L \cdot 12L$. This trisomic resembles triplo-12 in about half of its characters. Its growth habit is somewhat like triplo-12, but it is not as compact. Leaves are curled but the characteristic fimbriation of the leaves of triplo-12 is absent. The colour of leaves is deep grey-green instead of the bluish-grey of triplo-12. Leaves are broader, but not so serrate. It differs from triplo-12 in having an elongate inflorescence, but resembles it in having a reduced number of flowers per inflorescence. The pedicel is elongate. This trisomic has an extreme tendency towards blindness. Almost 90 per cent. of the seedlings go blind after producing one or two leaves. If left undisturbed in the seed pan, about 75 per cent. of them later produce axillary shoots, the remaining 25 per cent. not producing any further growth. This tendency for blindness is evident in the mature plants, as many shoots lose their growing points. Due to this inherent defect, the vegetative growth is one-half to one-third that of

triplo-12. Some flowers have normal anthers while in others one to five anthers may be degenerated. The anthers show varying degrees of adnation with the ovary. The green and ripe fruits may thus show one to five suture-like markings on the surface.

From the above morphological descriptions of the secondary trisomics, the following conclusions can be drawn:

(*a*) The long arms of chromosomes with sub-median centromeres exert a disproportionately greater influence than the short arms on the morphology of trisomic plants. The same conclusion was drawn from the study of telotrisomics (Khush and Rick, 1967*d*) and tertiary trisomics (Khush and Rick, 1967*e*). All of the morphological features of the related primary trisomic can, in fact, be easily seen in the telotrisomics and secondary trisomics for long arms.

(*b*) The secondary trisomics for short arms, like short arm telotrisomics, show scarcely any resemblance to the related primary trisomic. Most of the characters by which the secondary trisomics and telotrisomics for short arms differ from the diploids are therefore unpredictable. Since the differences from 2n sibs are small they can be distinguished only with difficulty.

(*c*) The secondary trisomic for the long arm of the metacentric chromosome 12 shows about half of the characters of the related primary trisomic and in general looks quite different from it. It therefore appears that the two arms of this chromosome contribute about equally to the morphology of the primary trisomic. The same may be true for the other two metacentric chromosomes of the complement, 5 and 11. The secondary trisomic for the short arm of metacentric chromosome 5 of maize (Rhoades, 1933) showed a similar relation to its primary.

(*d*) A finding of special interest that has emerged from the morphological examination of the secondary trisomics is that some primary trisomic characters result from the interaction of the two arms, which when present alone influence the morphology in opposite directions. Thus, 3S acts to produce pubescent stems and inflorescences in contrast to the glabrous stems and inflorescences conditioned by 3L. Similar relations can be revealed by comparing the morphology of $2n+7S \cdot 7S$ and $2n+7L \cdot 7L$ with that of $2n+7S \cdot 7L$ and the same for $2n+9S \cdot 9L$. Compared with the diploid, $2n+7S \cdot 7L$, for example, has thick stems, broad leaves, short internodes and compact inflorescences, which result in a bushy growth habit. All these characters are magnified in $2n+7L \cdot 7L$, which has even thicker stems, broader leaves, shorter internodes, more compact inflorescences and bushier growth habit. In contrast, the morphology of $2n+7S \cdot 7S$ is modified in the opposite direction. This trisomic has slender stems and leaves, longer internodes and inflorescences, and an open growth habit. Similarly, all the plant parts of $2n+9S \cdot 9L$ including internodes, leaves, flowers and fruits are somewhat smaller than the diploid, and in $2n+9L \cdot 9L$ they are even smaller. However, in $2n+9S \cdot 9S$ all these organs are bigger than in the diploid. It is therefore evident that the primary trisomics are intermediate between the two related secondaries in several characters. A similar relationship was found for several primary and secondary trisomics of *Datura stramonium* by Blakeslee and his associates (Avery, Satina and Rietsema, 1959, p. 89).

5. GENETICS

The genetic ratios are modified in the same fashion in the progenies of secondary trisomics as in the progenies of telotrisomics (Khush and Rick, 1967d) and tertiary trisomics (Khush and Rick, 1966b, 1967e). The nature of the modified ratios permits the determination of arm locations of markers. All the gametophytes that receive an isochromosome in place of a normal chromosome abort because one arm is deficient; consequently, the n gametes and resultant 2n zygotes produced by secondaries can only consist of normal homologues. Therefore, if a secondary trisomic carries a recessive marker on one normal chromosome and the dominant allele on the normal homologue, the diploid fraction of its F_2 and BC progeny should segregate 3:1 and 1:1, respectively. All of the secondary trisomic progeny should have the normal phenotype if the marker has its locus on the isochromosome. If the marker is not located on the duplicated arm, normal disomic ratios should obtain for the secondary trisomic fraction. Observed ratios, whether 3:1::all:0 or 3:1::3:1 for the F_2; or 1:1::all:0 or 1:1::1:1, in the BC, can thereby identify the appropriate arm for the marker. In the telotrisomic and tertiary trisomic progenies giving trisomic segregations, an occasional trisomic individual with recessive phenotype may be obtained by double reduction. Such individuals were in fact obtained in the progenies of several tertiary trisomics and telotrisomics. However, if a secondary trisomic progeny segregates in a trisomic fashion, no secondary trisomic with recessive phenotype should appear, as only one arm, but not both, of the isochromosome can receive the recessive allele by crossing over. Thus, as soon as a secondary trisomic with recessive phenotype is observed, it can be safely concluded that the progeny is segregating in disomic fashion and that no further scoring is necessary.

As will be discussed in the next section, the progenies of most of the secondary trisomics regularly include related primary trisomics, all of which should have normal phenotype if the marker is situated very near the centromere. If the marker is located away from the centromere, however, an occasional primary trisomic with the recessive phenotype may be obtained. Thus, if an appreciable number of primary trisomics appear in the segregating secondary trisomic progeny, approximate location of the marker in relation to the centromere can be determined. Moreover, by studying the genetic ratios among the primary trisomic fraction of the progeny, chromosomal location of the marker can be verified.

From the foregoing discussion, it is evident that three kinds of ratios may be obtained among the progenies of most of the secondary trisomics. The ratio among the diploid fraction should vary from disomic for genes near the centromere to lower values for those more distally located. For a heterozygote with a recessive gene on one of the normal chromosomes and the dominant allele in the three other homologous arms, the minimal gametic ratio expected of a locus enjoying free recombination between the four arms would be 3:1. Among the primary trisomic fraction, the ratio should be trisomic if the marker is located on that chromosome. Finally, the secondary trisomic fraction should give trisomic ratios if the marker is located on the isochromosome arm and disomic ratios if it is located on the other arm. Therefore, those secondary trisomics which yield an appreciable number of primary trisomics in their progenies can be utilised, like compensating

trisomics (Khush and Rick, 1967*a*), for simultaneous assignment of unlocated markers to their respective chromosomes and arms.

According to Avery, Satina and Rietsema (1959), the secondary trisomics of *Datura stramonium* were utilised for determining the arm location of several markers. As far as we are aware, however, no published data on the secondary trisomic ratios obtained in *Datura stramonium* or any other species are available. Blakeslee (1924) presented data on the segregating progenies of two secondary and the two related primary trisomics. Both of the primary trisomics gave trisomic ratios with their respective genes, but, according to the author, only one secondary gave trisomic ratios, not the other. These data are unsatisfactory for the following reasons.

Firstly, one of the two supposed secondaries, wiry, was later found to be a tertiary trisomic. Secondly, the cytological nature of the secondary trisomic at that time was not clear, and Blakeslee supposed that the ratios for the primary and secondary trisomics should be similar—a supposition that we now know for reasons outlined above to be erroneous. Thirdly, for the sake of " convenience ", he presented data on the diploid fraction of the progenies only. We know now that ratios in the diploid portions of the progenies of secondary and tertiary trisomics should remain unmodified. These data are therefore useless. Moreover, he obtained an 8:1 ratio for *p* in the diploid portion of progeny of " poinsettia " (a primary trisomic) as well as in the diploid portion of the progeny of " wiry " (a tertiary trisomic). Such a ratio is expected for the former trisomic, but not for the latter. Similarly, he obtained a ratio of 8:1 for *a* in the diploid portion of " cocklebur " (a primary) and a ratio of 3:1 for the same gene in the diploid fraction of " wedge " (a related secondary). The trisomic nature of the former segregation is unquestionable, but his interpretation of the diploid nature of the latter is invalid. All of the secondary trisomics of the progeny may have had normal phenotype with respect to *a*, and the ratio may have been trisomic.

In view of the above considerations, the data from the segregating progenies of secondary trisomics of tomato can be analysed to yield information about the chromosomal and arm location of the markers and their approximate distances from the centromere. Five secondary trisomics were used to study segregation of 10 genes belonging to four chromosomes of the complement (table 3). Of the remaining four secondaries, $2n + 6L \cdot 6L$ does not transmit, thus F_1 trisomics could not be obtained, and the other three were obtained so recently that they have not yet been employed in genetic studies. As may be seen from table 3, the diploid portions of all of the progenies gave, as expected, essentially disomic ratios. Among the primary trisomic fractions all the markers except *lg* gave trisomic ratios. Since *lg* segregated in diploid fashion among the primary trisomic fraction, the previous assignment of this marker by linkage test (Butler, 1954) to chromosome 10 must have been in error. Three triplo-10's with *ag* phenotype were produced by double reduction, indicating thereby that *ag* must be distant from the centromere. In the same family, all the triplo-10's were h^+, thus suggesting that *h* is closer to the centromere than *ag*. Similarly, since one triplo-12 with *alb* phenotype, but none with *fd*, was obtained, it appears that *alb* is more distal from the centromere of 12 than *fd*. However, the number of primary trisomics in both of the families was so small that the foregoing conclusion must at best be considered tentative. The data of table 3 show that all the genes except *ag* and *h* segregated in disomic fashion among the secondary trisomic

TABLE 3

Genetic ratios in the backcrosses of secondary trisomics

Trisomic	Gene	Total	Diploid			Parental secondary trisomic			Related primary trisomic			Unrelated 2n+1
			Normal	Recessive	% Recessive	Normal	Recessive	% Recessive	Normal	Recessive	% Recessive	
2n+8L·8L	re	268	114	102	47·2	9	6	40·0	34	0	0·0	3
2n+9S·9S	pum	298	119	115	49·1	44	17	27·8	3	0	0·0	0
	ah	298	119	115	49·1	44	17	27·8	3	0	0·0	0
	marm	298	119	115	49·1	38	23	37·7	3	0	0·0	0
2n+9L·9L	wd	397	147	153	51·0	37	30	44·7	29	0	0·0	1
2n+10L·10L	h	392	136	126	48·0	78	0	0·0	50	0	0·0	2
	ag	392	162	100	38·1	78	0	0·0	47	3	6·0	2
	lg	289	98	87	47·3	26	26	50·0	23	29	55·7	0
	t	134	62	57	48·0	15	0	0·0				
	u	134	55	64	53·8	11	4	26·7				
2n+12L·12L	alb	300	121	128	51·4	24	18	42·8	8	1	11·1	0
	fd	294	122	125	50·6	25	14	35·9	8	0	0·0	0

Progeny

fractions. Probably because of reduced viability, the number of secondary trisomics with recessive phenotypes was less than 50 per cent. in most of the families giving disomic ratios. But there was hardly any doubt about the disomic inheritance in these families. All of the secondary trisomics of the family segregating for *ag* and *h* had normal phenotype for these genes, and the trisomic nature of this segregation is clear.

The inheritance data with secondary trisomics presented above have increased our understanding of at least four linkage groups as follows.

The arm locations of three genes of the linkage group *l—ms-8—re—cpt—bu—ch—dl—al* of chromosome 8 had previously been determined by means of radiation-induced deficiencies (Rick and Khush, 1966), *l* allocated to 8S and *bu* and *dl* to 8L. Additionally, *cpt* was positioned to 8L by means of a telotrisomic test. The exact position of the centromere was not known, although it was understood that it must lie somewhere in the *l—cpt* interval. Because *re* gave a disomic ratio with 2n + 8L·8L (table 3) in the present studies, it must lie on 8S, and the centromere can now be mapped between *re* and *cpt*. The closeness of *re* to the centromere is evident from the chromosome type segregation obtained for this gene among the primary trisomic fraction of the progeny.

The two markers, *nv* and *ah*, of the linkage group for chromosome 9 had been located to 9L by means of radiation-induced deficiencies (Khush, Rick and Robinson, 1964), and *marm* had been located to 9L by means of a tertiary trisomic test (Khush and Rick, 1967e). To determine the arm location of *pum*, 2n + 9S·9S was made heterozygous for the marker group *pum—ah—marm*. As the data of table 3 show, all three genes gave disomic ratios among the secondary trisomic subpopulations. These results confirm the previous locations of *ah* and *marm* and limit *pum* to 9L as well. One marker of the Verkerk series (No. 377-20*αα*) was found to be closely linked to *ah* by Robinson (1966). Seeking a marker for 9S, we tested this gene with 2n + 9L·9L, and, as the data of table 3 show, it gave disomic ratios and must therefore lie on 9S. The chromosomal location of this gene can be verified from the trisomic ratio obtained in the primary trisomic fraction of the progeny. Being identical in phenotype with *wd*—another marker of 9 which is known to be closely linked to *ah*—this new marker is suspected to be identical or allelic with it.

In the latest chromosome map of tomato (Clayberg, Butler, Kerr, Rick and Robinson, 1966), the linkage group of chromosome 10 is shown to be *lg—pe—u—oli—h—1-2—t—ag—Xa*. The markers *t*, *ag* and *Xa* were previously located on 10L by means of a tertiary trisomic test (Khush and Rick, 1967e), but these determinations did not reveal the orientation of the linkage map or the position of the centromere. To accomplish this purpose, *lg*, *h* and *u* were tested against 2n + 10L·10L. As discussed already, *lg* gave disomic segregation among the triplo-10 fraction of the segregating progeny of 2n + 10L·10L (table 3) and thus cannot be situated on chromosome 10. Butler (1954) reported *lg* and allocated it to chromosome 10. The latest map distance between *pe* and *u* was reported as 43 cM by Clayberg, Butler, Kerr, Rick and Robinson (1966). *Bona fide* linkages of the latter magnitude are notoriously difficult to verify. It is therefore evident that neither *pe* nor *lg* is located on chromosome 10 and that the reported linkage between *pe* and *u* must be spurious. On the other hand, *h* gave trisomic ratios amongst secondary trisomic as well as primary trisomic subfamilies and must therefore

be located on 10L. This secondary trisomic family was also segregating for *ag*, and trisomic ratios were obtained as expected for this marker. The fact that double reduction was observed for *ag* but not for *h* amongst the triplo-10 subfamily argues for a proximal position of *h* and distal for *ag* on 10L. The map must therefore be oriented as indicated above, but with *u* at the 0 position instead of *lg*. A backcross test between *u* and $2n+10L \cdot 10L$ segregated disomically for *u* in the diploid and secondary trisomic fractions of the population (table 3), hence *u* must reside in 10S and the centromere between *u* and *h*. In the same progeny, *t* segregated in trisomic fashion amongst $2n+10L \cdot 10L$ individuals as expected. The greenhouse environment of this test did not permit detection of triplo-10, whereas the phenotype of $2n+10L \cdot 10L$ was distinct.

Additional evidence of a distal locus for *ag* is provided by its segregation in the diploid fraction. The observed proportion of *ag/ag* (38·1 per cent.) is significantly lower than expected ($\chi^2 = 14 \cdot 2$***) for a locus close to the centromere and closely approximates the 35 per cent. expected for a distant, freely recombining locus if account is taken of the fact that 60 per cent. of the cells of this trisomic were observed to form trivalents.

Only two genes have been located on chromosome 12 to date: *alb* was delimited to 12S (Khush and Rick, 1966a) and *fd* to 12L (Rick, Khush and Andrásfalvy, 1967) by means of radiation-induced deficiencies. The two arms of chromosome 12 are so similar in length and chromomere pattern that it is difficult to distinguish between them in cytological preparations. Being thus somewhat uncertain of the arm location of *fd*, we tested *fd* as well as *alb* with $2n+12L \cdot 12L$. Since both gave disomic ratios in the secondary trisomic fractions of their progenies, they must lie on 12S. Although the primary trisomic fraction of each family was very small, the fact that a recessive homozygote appeared for *alb* but none for *fd* (table 3) suggests that *fd* might be proximally located to *alb*.

6. TRANSMISSION RATES

The data on the transmission rates of seven secondary trisomics are presented in table 4. The progenies of the remaining two secondaries, $2n+7L \cdot 7L$ and $2n+3S \cdot 3S$, have not yet been grown as they were obtained only recently. For five of these trisomics, the data on transmission rates have been calculated from the data of table 3, while for $2n+6L \cdot 6L$ and $2n+7S \cdot 7S$ backcross progenies with cv. VF36 as the male parent were grown. It should be noted that all the progenies from which these data were taken had high heterozygosity, which tends to enhance transmission rates of extra chromosomal types.

As may be seen from table 4, the transmission rates of secondary trisomics varied between 0·0 per cent. for $2n+6L \cdot 6L$ and 33·6 per cent. for $2n+7S \cdot 7S$. The tremendous difference between the transmission rates can be explained on the basis of differential unbalance caused by the extra isochromosomes. Extra $6L \cdot 6L$ causes so much unbalance that none of the $n+6L \cdot 6L$ gametophytes function. Similarly, the viability of $n+8L \cdot 8L$ gametophytes appears to be very low. In general, the extra isochromosomes of long arms cause greater unbalance than those of short arms.

The proportion of related primary trisomics in the progenies of tomato secondaries varies widely (table 4) like the classical ones of *Datura stramonium*.

No primaries appeared in the progeny of 2n + 7S·7S, but a maximum of 14·9 per cent. was observed in the progeny of 2n + 10L·10L. The corresponding range for *Datura* primaries was 0·06 per cent. in the progeny of 2n + 19·19 to 10·01 per cent. in the progeny of 2n + 9·9 (Avery, Satina and Rietsema, 1959). The ratio of related primaries to the parental secondaries was also interestingly variable. In the progeny of 2n + 6L·6L, for example, only primaries but no secondaries appeared; for 2n + 7S·7S the opposite was obtained. The proportion of primaries was more than double the proportion of secondaries in the progeny in 2n + 8L·8L, while it was lower in the progenies of other secondaries (table 4). The cytological requirements which

TABLE 4

Transmission rates of secondary trisomics

			Progeny				
			Parental 2n+1		Related primary 2n+1		Unrelated*
Trisomic	Total	2n	No.	%	No.	%	2n+1
2n+6L · 6L	681	510	0	0·0	71	10·4	0
2n+7S · 7S	259	170	87	33·6	0	0·0	2
2n+8L · 8L	268	216	15	5·6	34	12·7	3
2n+9S · 9S	298	234	61	20·4	3	1·0	0
2n+9L · 9L	397	300	67	15·3	29	7·3	1
2n+10L · 10L	681	447	130	19·1	102	14·9	2
2n+12L · 12L	594	496	81**	15·3	17	2·8	0

* Chromosomal constitution of unrelated trisomics is given in the text.
** Total does not include 40 blind secondary trisomics.

give rise to n + 1 gametes having a primary extra chromosome instead of a secondary were discussed in the section on cytology. The correlation between low frequency of trivalent formation in 2n + 7S · 7S and 2n + 9S · 9S on the one hand (table 2) and the low frequency of the primaries in their progenies on the other (table 4) is clear.

A few unrelated primary trisomics appeared among the progenies of some of the secondaries (tables 3 and 4). 2n + 7S·7S yielded two triplo-4; 2n + 8L·8L, one triplo-10 and two triplo-7; 2n + 9L·9L, one triplo-5; 2n + 10L·10L, one triplo-2 and one triplo-12. These trisomics undoubtedly appeared as a result of non-disjunction in other members of the complement. The results suggest that the presence of extra isochromosomes might increase the spontaneous rates of non-disjunction in unrelated chromosomes.

7. DISCUSSION

Of the group of nine secondary trisomics of tomato reported in this paper, ?n + 6L·6L is probably most interesting. This trisomic originated sponta-..eously in the progeny of haplo-4S·10S. The plant had good vigour, flowered profusely and set a moderate fruit load. When a large progeny was raised, the parental trisomic did not appear, only diploid and triplo-6. Clearly gametophytes that receive an extra 6L·6L isochromosome do not tolerate such unbalance. It was shown earlier (Khush and Rick, 1967b) that gametophytes carrying extra 2L·2L or the 3L·3L isochromosomes do not

survive either. The gamete which gave rise to the $2n + 6L \cdot 6L$ plant was probably produced by a gametophyte carrying an extra 6L telocentric, which was converted into an isochromosome either during gametogenesis or during the early ontogeny of the sporophyte. Thus $2n + 2L \cdot 2L$ and $2n + 3L \cdot 3L$ plants may also appear in this same fashion, but, like $2n + 6L \cdot 6L$, they will never transmit the extra isochromosome to the next generation. On the basis of arm length, the same is likely to hold for $2n + 1L \cdot 1L$ and $2n + 4L \cdot 4L$.

Thus all the 24 possible secondary trisomics may be viable sporophytes, but the gametophytes carrying an extra $1L \cdot 1L$, $2L \cdot 2L$, $3L \cdot 3L$, $4L \cdot 4L$ or $6L \cdot 6L$ probably do not survive. Transmissible secondary trisomics for the other 19 arms should be obtainable. Ten are already known: nine reported here and one by Moens (1965).

Compared to such other genetically well-known plant species as maize, the tolerance limits for deficiencies and duplications in tomato are narrower. In maize, monosomics for four different chromosomes (Baker and Morgan, 1966), including the longest chromosome which measures $82 \cdot 4\mu$ or $14 \cdot 9$ per cent. of the total length of the complement, have been obtained. The longest deficiency obtained in tomato is that of chromosome 11 which measures $24 \cdot 8\mu$ or $6 \cdot 4$ per cent. of the total length of the complement. At the haploid level, no euchromatic deficiency, and only one small heterochromatic deficiency, have been found to survive in tomato. In maize, however, many small deficiencies of euchromatic regions are known to survive and transmit to the next generation (McClintock, 1941, 1944; Patterson, 1952). Even rather large deficiencies in maize comprising one-fourth of the chromosome arm have transmitted to the next generation through the female side (Stadler, 1933, 1935).

The maximum number of extra chromosomes tolerated by the sporophytes of tomato is three (Rick and Barton, 1954), and, in these triple trisomics, only the smaller chromosomes of the complement are found to be extra. In maize, plants with six to seven extra chromosomes are known to be viable (McClintock, 1929). Thus, maize tolerates higher levels of duplication and deficiency than the tomato.

Differences in tolerance for deficiencies and duplications are usually explained in terms of levels or degrees of polyploidy. The comparison of experimental evidence available for maize and tomato therefore indicates that the latter is even more " diploid " than the former. This conclusion is also supported by observations of lowered phenotypic effect of extra chromosomes and more frequent occurrence of duplicate genes in maize than in tomatoes.

8. SUMMARY

1. Nine different secondary trisomics of the tomato were investigated. Three originated from haplo-triplo-disomics, three from double iso-compensating trisomics, two from tertiary monosomics, and one arose independently on three occasions from different kinds of deficiencies. The most productive source in our experience is karyotypes that possess isochromosomes which were obtained by induced breakage and reunion of the centromere.

2. Chromosomal associations were studied at pachytene and diakinesis, and relative frequencies of various diakinetic configurations were found to

Photomicrographs of tomato pachytene chromosomes × 2000.

Fig. 1.—Internally paired isochromosome 3S · 3S in 2n+3S · 3S.

Fig. 2.—Trivalent association in 2n+3S · 3S. Only heterochromatic part of 3L is visible.

Fig. 3.—Trivalent association in 2n+6L · 6L. Only about the proximal half of paired 6L's can be seen.

Fig. 4.—(*a*) Internally paired 6L · 6L isochromosome in 2n+6L · 6L.
(*b*) Interpretive drawing of a.

Fig. 5.—Trivalent association in 2n+7S · 7S.

Fig. 6.—Trivalent association in 2n+8L · 8L. One chiasma can be easily seen in the 8L's.

Fig. 7.—Internally paired isochromosome 8L · 8L in 2n+8L · 8L.

Fig. 8.—Internally paired isochromosome 7S · 7S in 2n+7S · 7S.

Fig. 9.—Internally paired isochromosome 9S · 9S in 2n+9S · 9S.

Fig. 10.—Trivalent association in 2n+9S · 9S.

Fig. 11.—Trivalent association in 2n+9L · 9L. One chiasma can be easily seen in the 9L's.

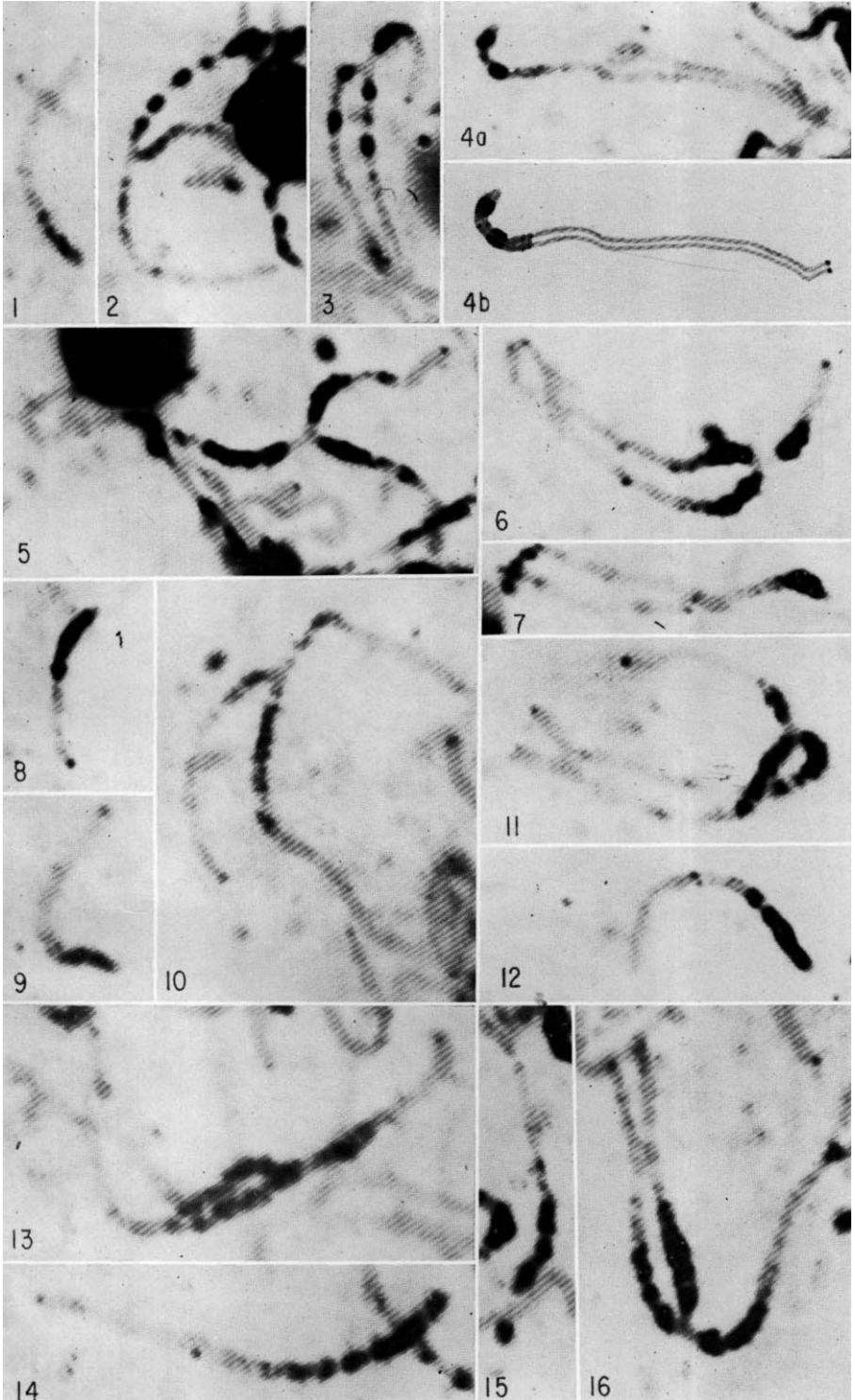Fig. 12.—Internally paired isochromosome 9L · 9L in 2n+9L · 9L.
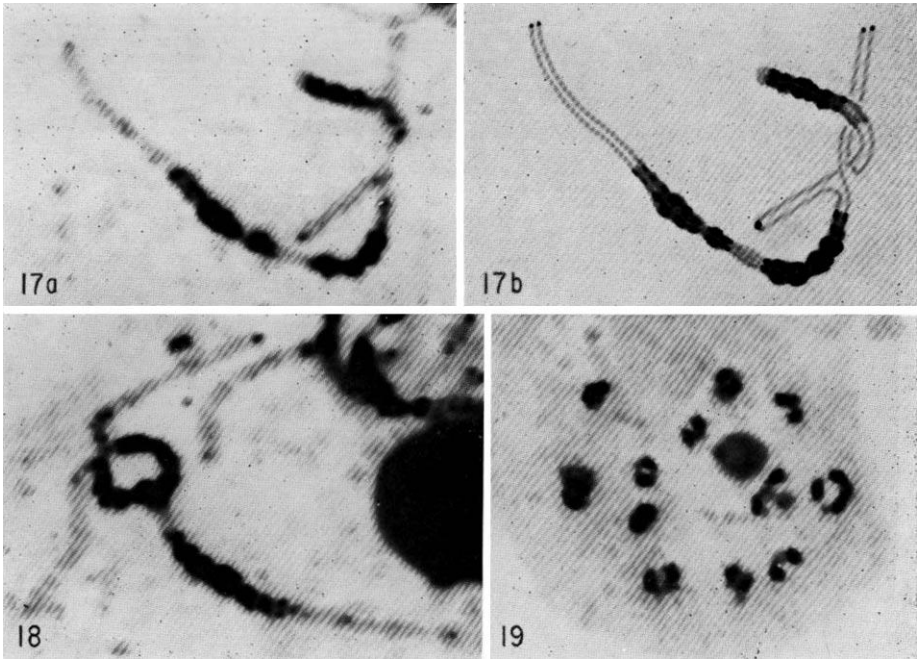
Fig. 13.—Trivalent association in 2n+10L · 10L. Two chiasmata can be seen in the 10L's.

Fig. 14.—Internally paired isochromosome 10L · 10L in 2n+10L · 10L.

Fig. 15.—Internally paired isochromosome 12L · 12L in 2n+12L · 12L.

Fig. 16.—Trivalent association in 2n+12L · 12L. One chiasma can be seen in the 12L's.

Plate II

Photomicrographs of tomato chromosomes. Figs. 17 and 18 at pachytene stage of meiosis × 2000; fig. 19 at diakinesis × 600.

FIG. 17.—(*a*) Trivalent association in $2n+12L \cdot 12L$. (*b*) Interpretive drawing of *a*.

FIG. 18.—Trivalent association in $2n+12L \cdot 12L$. One chiasma can be seen in the 12L's.

FIG. 19.—11 bivalents and 1 ring trivalent in $2n+10L \cdot 10L$.

agree roughly with expectations based on arm lengths and number of chiasmata. When univalent, the isochromosomes tended to pair internally, suffer chiasmata, and form ringlets. The low frequencies of trivalent formation in $2n+7S \cdot 7S$ and $2n+9S \cdot 9S$ led to the low yield of corresponding primary trisomics in their progeny.

3. The transmission rates of isochromosomes varied greatly, none having been recovered from $2n+6L \cdot 6L$ and few from $2n+8L \cdot 8L$, presumably because such imbalance is at the limit of gametophytic tolerance in tomatoes. For the same reason no haplo-triplo-disomic progeny were yielded.

4. Comparisons of gross morphology between secondaries and their corresponding primaries revealed that the effect wrought by long arms is disproportionate to their relative length while that by short arms is generally unpredictable. The two arms may exert an opposing influence on the same character, in which case the effect of the long arm always dominates that of the short one.

5. The first inheritance data for monogenic segregation in secondary trisomics of any organism are presented. For those secondaries that yield corresponding primaries in their progeny, such segregations can identify the chromosome on which the gene in question is located, the arm and the relative distance from the centromere. Information was thereby obtained for four tomato chromosomes to clarify the relationships between chromosomes and their genetic linkage maps. For chromosomes 8, 9 and 10 the centromere positions could be placed more precisely on the maps, and for chromosome 10 a previously reported linkage was proven to be spurious.

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