Tomato Telotrisomics: Origin, Identification, and Use in Linkage Mapping[1]

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Derived telocentric chromosomes, which consist of the centromere and one complete arm of a normal chromosome, have been reported in several species. A telocentric chromosome may replace a normal, homologous chromosome or may be present in addition to the diploid complement. Sears (1954) has reported many examples of the former type in hexaploid wheat, *Triticum aestivum*, and named them monotelosomes. Such aneuploids are of great value for their revealing in polyploid species the arm location of genes by means of monosomic analysis. Monotelosomes have been used thus in wheat (Sears 1954, 1962), cotton (White and Endrizzi 1965, Endrizzi and Kohel 1966), and oats (McGinnis, Andrews, and McKenzie 1963). In diploid species monotelosomes cannot be used for monosomic analysis because they are not transmitted to the next generation. If present as additions to the normal diploid complement, however, they are inherited and can be used for determining arm locations by means of trisomic analysis. Such individuals have been designated " telosomic trisomics " by Burnham (1962), hereafter abbreviated by us to " telotrisomics ".

Telotrisomics have been reported in several species including *Zea mays* (Rhoades 1936), *Datura stramonium* (Blakeslee and Avery 1938), *Nicotiana sylvestris* (Goodspeed and Avery 1939), *Triticum monococcum* (Moseman and Smith 1954), *Hordeum vulgare* (Tsuchiya 1960), and *Secale cereale* (Kamanoi and Jenkins 1962). Rhoades (1936) employed a telotrisomic of the short arm of chromosome 5 in maize for finding the arm location of several markers of the linkage group on this chromosome. Similarly, Moseman and Smith (1954) used a telotrisomic of *Triticum monococcum* for determining the arm location of two markers. In most of these species the problems of identifying the telocentric arms limit their usefulness. In the tomato (*Lycopersicon esculentum*), however, thanks to cytologically detectable individuality of the pachytene chromosomes, the telocentric arms can be identified and have proved useful in cytogenetic analysis. The present paper deals with the origin, identification, and cytogenetics of six telotrisomics of the tomato.

Origin and cytology

Until now six telotrisomics of the tomato have been discovered--namely, 2n+3L, 2n+4L, 2n+7L, 2n+8L, 2n+3S and 2n+10S. One, triplo-4L, was synthesized intentionally ; the others appeared spontaneously in our cultures. The pistillate parent of triplo-4L was entirely deficient for 4S, but had translocated to the centromere of its 4L a large heterochromatic chromomere, probably from the satellite region of 2S (Khush and Rick 1967c). A cross between this plant and a normal diploid yielded 1,601 progeny including two desired 2n+4L, which were obviously generated by gametes that received by
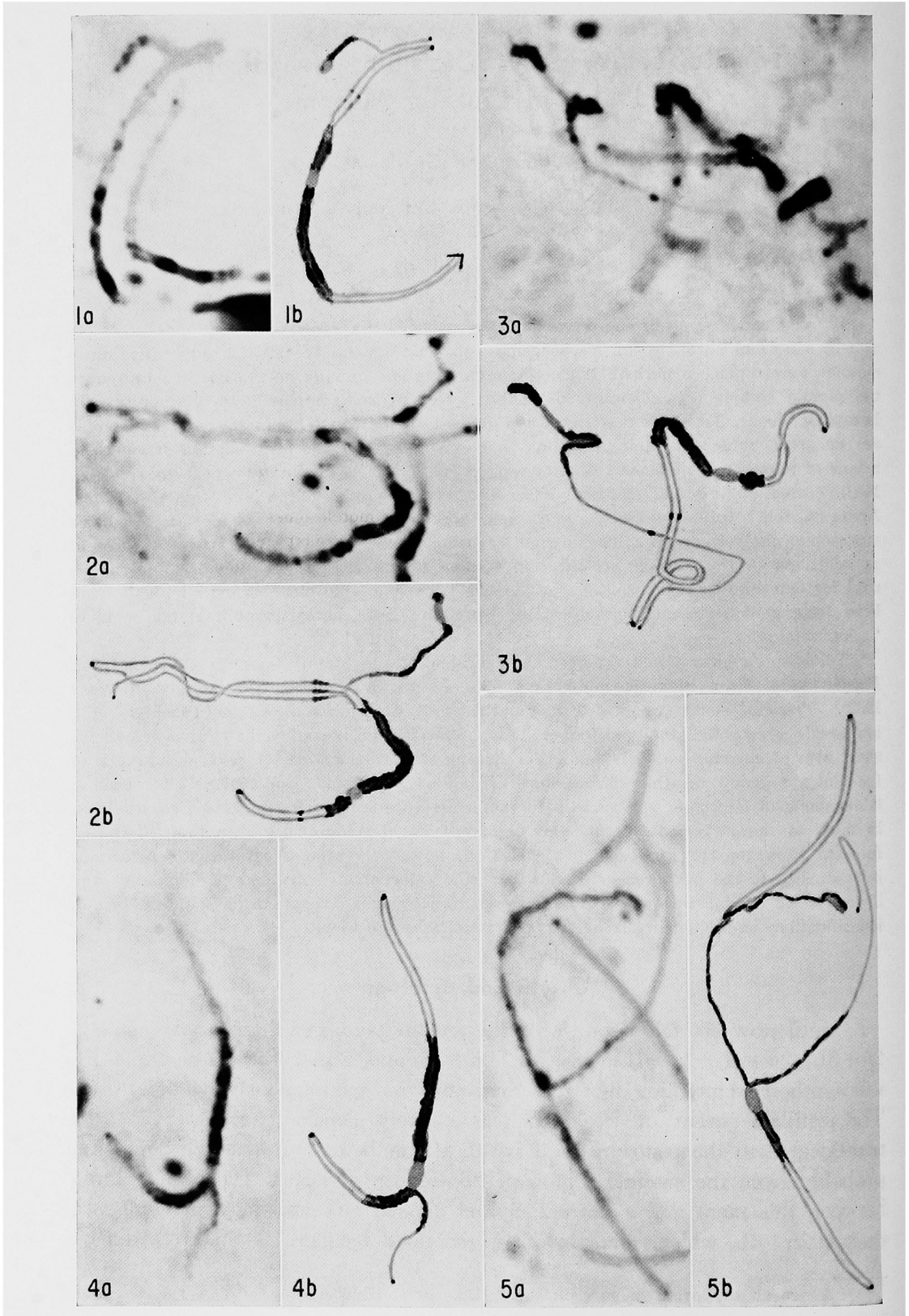
nondisjunction the telocentric 4L in addition to the normal complement. The centromere of 4L in this telotrisomic therefore is not terminal (Fig. 3).

Triplo-3L appeared spontaneously in the progeny of the tertiary monosomic haplo-3S-11L (Khush and Rick 1966a). The centromere of telocentric 3L is also subterminal, as a heterochromatic chromomere of unknown origin replaces 3S (Fig. 2). In all probability the telocentric originated from the fracture of the tertiary chromosome 3L·11S of the parent. Triplo-8L similarly appeared in the progeny of a tertiary monosomic, haplo-8S-5L. The centromere of 8L is likewise not terminal, but a small heterochromatic knob appears on the other side of the centromere from 8L (Fig. 6). Triplo-7L was found in the progeny of the aforementioned haplo-3S-11L, and the centromere of this telosome appears to be completely terminal (Fig. 5). Triplo-3S, which has a terminal centromere (Fig. 1), was yielded by a compensating trisomic in which a normal chromosome 3 was replaced by a telocentric for 3S and an isochromosome for 3L (Khush and Rick 1967a). Finally, triplo-10S, which also possesses a terminal centromere (Fig. 4), appeared spontaneously in the progeny of haplo-2S-triplo-2L (Khush and Rick 1967b).

It is noteworthy that three of the telocentrics, 3S, 7L, and 10S, have what appear to be truly terminal centromeres, those of 7L and 10L apparently of normal size, that of 3S about half normal size. The other three, 3L, 4L, and 8L, have subterminal centromeres with one heterochromatic chromomere apiece instead of the respective short arm. According to their morphology, these chromomeres do not belong to the missing arms, but seem to have been translocated from nonhomologous chromosomes. Technically, therefore, the latter three telotrisomics might be classified as tertiary trisomics, but a single heterochromatic chromomere constitutes such an insignificant (and probably inert) part of an entire arm that it might be safely ignored—hence our preference for the telotrisomic classification.

Among the six telosomics examined, the telocentric chromosomes either paired with the normal homologues to form a trivalent or remained unpaired as univalents. At pachytene the telocentrics for the long arms were observed as univalents in very few cells, while those for short arms were quite fre-

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Figs. 1-5. Tomato chromosomes in pachytene of the first meiotic division showing configurations of various telotrisomics. Of the pair of illustrations in each figure, a) is the photomicrograph, b) an interpretive drawing. ×2,000. 1, telotrisome for 3S. Chromatin of the telocentric 3S is strongly condensed in contrast to that of the two normal chromosomes. Centromere of the telocentric is folded back on 3S and out of focus. Arrow points in the direction toward which the long arm could not be traced with certainty. 2, telotrisome for 3L. Note the subterminal centromere of the telocentric in the upper right and trivalent association of strands in center. 3, telotrisome for 4L. Note the subterminal centromere and nonhomologous association of heterochromatin in the telocentric. 4, telotrisome for 10S. The centromeres of the three chromosomes are paired to form a single unit. 5, telotrisome for 7L. The telocentric is unpaired for its heterochromatic region and the terminal centromere is folded back and lies in a different focal level than that of the photomicrograph.

quently univalent. The extra telocentric can be easily identified at pachytene either from the morphology of the univalent itself or from that of the
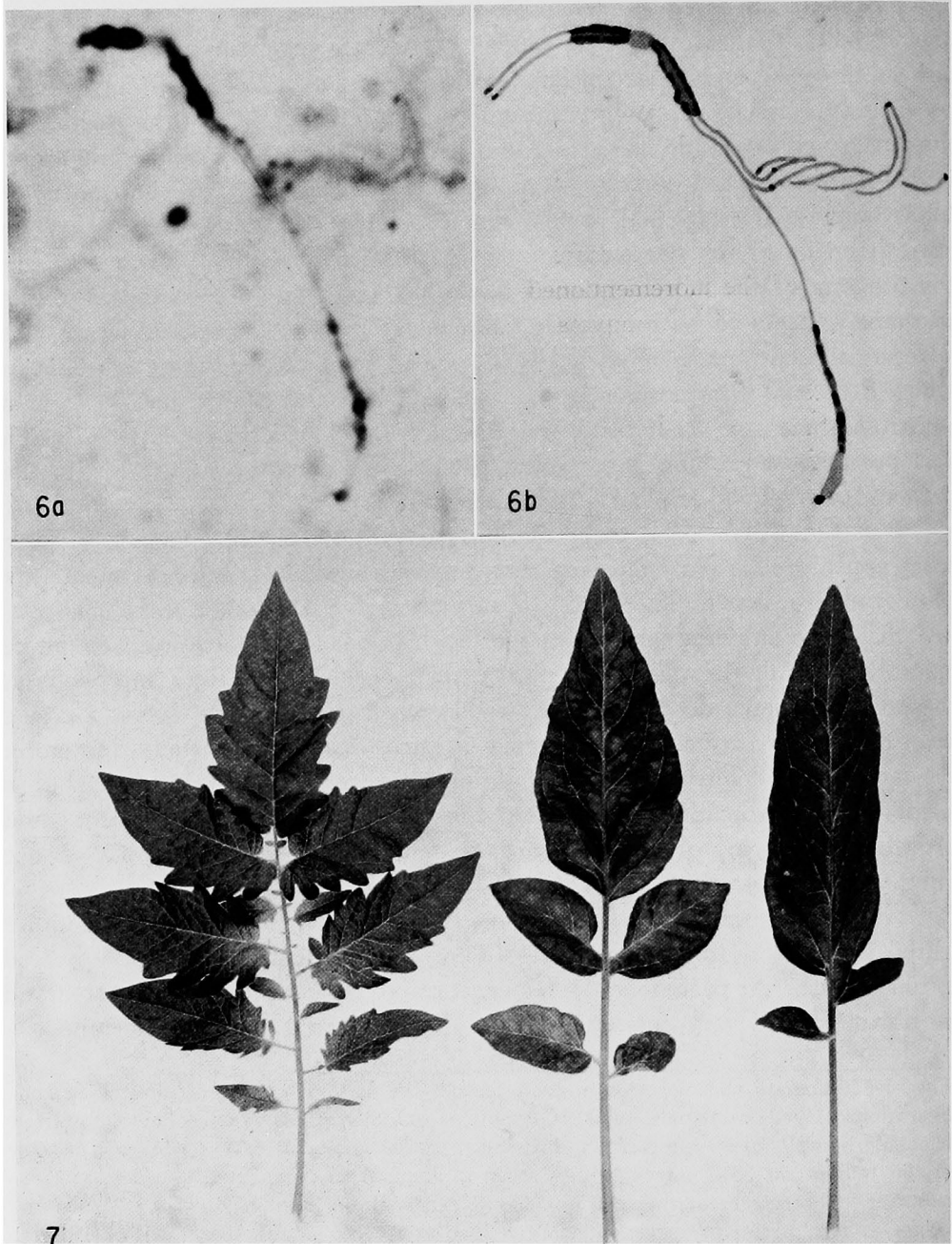
Figs. 6-7. 6, tomato chromosomes in pachytene of the first meiotic division showing a figure of the telotrisome for 8L. In this group, a) is the photomicrograph, b) an interpretive drawing. Note the subterminal centromere of the telocentric and trivalent association of strands for part of the euchromatin of 8L. The univalent portion of the telocentric is stretched out of proportion. ×2,000. 7, representative mature leaves, upper surface. Left, normal (+/+); center, triplo-7L with one dose of *La(La/+/+)*; right, diploid heterozygous for *La(La/+)*. ×1/2.

trivalent. The trivalents of all the six telotrisomics are shown in photo-micrographs of Figs. 1–6.

At diakinesis either a trivalent and eleven bivalents or one univalent and twelve bivalents were observed. Rarely a microsporocyte with three univalents and eleven bivalents appeared. The frequency of different associations observed at diakinesis is summarized in Table 1. Trivalents were more frequent in the telotrisomics for long arms, while the telotrisomics for short arms tended to form more univalents. This tendency is predictable on the basis of length alone since the longer telocentrics have more chance than the shorter ones to pair and form chiasmata with the normal homologues. The telocentrics 3S and 10S are so small in comparison with normal chromo-somes that the univalents of these telocentrics can be readily distinguished from normal chromosomes at diakinesis. In all microsporocytes examined of triplo-3S and triplo-10S with twelve bivalents and one univalent, the latter was always the telocentric. It therefore follows that the two normal chromosomes and the telocentric do not pair at random, but that the two

Table 1. Frequency of different associations of chromosomes at diakinesis in telotrisomics
(50 microsporocytes examined in each sample)

Telotrisomic	Per cent cells with		
	$11_{II}+1_{III}$	$12_{II}+1_{I}$	$11_{II}+3_{I}$
2n+3L	42	54	4
2n+4L	60	40	0
2n+7L	58	42	0
2n+8L	58	42	0
2n+3S	26	74	0
2n+10S	24	76	0

normal homologues pair preferentially with each other. Likewise the segrega-tion of the elements of these telosomic configurations at anaphase I seems to be nonrandom. Fifty microsporocytes each of triplo-3S and triplo-10S were examined at anaphase I for this purpose. The chromosomal distribution was invariably 12 : 12 + telocentric ; in no case was 13 : 11 + telocentric ob-served. This cytological behavior probably accounts for the rarity of related primary trisomics in the progenies of telotrisomics—a topic to be presented in a later section.

Five of the six telocentrics show normal cytological behavior in that they are somatically stable and neither lag nor misdivide to form isochro-mosomes. Only one, that of triplo-3S, is unstable and becomes lost in somatic tissues. Its somatic instability was first noted in the parental com-pensating trisomic, $2n-3S\cdot3L+\cdot3S+3L\cdot3L$ (Khush and Rick 1967a). A triplo-3S plant was derived in which the recessive chlorophyll suppressor *ru* was carried in (the short arm of) both of the normal 3 chromosomes. Thanks

to the presence of ru^+ on 3S, this plant had normal phenotype, except that somatic loss of 3S resulted in patches of ru phenotype on most of the leaves in the same fashion as in the parental compensating trisomic.

Gross morphology

It was consistently observed in this survey that the telocentrics for the long arms are strikingly similar in gross morphology to the corresponding primary trisomics, while those for short arms are nondescript, being distinguishable from diploids only under optimum conditions. Chromosome counts are therefore sometimes necessary to establish the trisomic nature of triplo-3S and triplo-10S. The telotrisomics for the two arms of chromosome 3 show different morphological characters of primary triplo-3. Triplo-3, for example, has slender as well as elongate plant parts. Its internodes, leaves, flower parts, and fruits are longer, but also narrower than those of diploids. On the other hand, the telotrisomic for 3L has slender but not elongate organs, while triplo-3S has elongate but not slender parts. Each arm of a chromosome evidently has a specific effect on morphology. Triplo-4L has finely divided leaves with recurved terminal segments like those of triplo-4. Triplo-7L resembles triplo-7 in having stocky habit and broader dark green leaves with upturned margins. Since triplo-7L is less hairy than triplo-7, the increased hairiness of the latter is probably controlled by 7S. Finally, triplo-8L resembles triplo-8 in its broad, wedge-shaped cotyledons, smaller leaves with convex segments, smaller flowers, exserted stigmas, and whitish unripe fruit.

Genetics

Genetic ratios in telotrisomic progenies are modified in the same fashion as those of tertiary trisomics (Khush and Rick 1966b). Complements in which a telocentric replaces a normal chromosome fail to survive gametogenesis; consequently, n gametes and 2n zygotes can be constituted only by normal homologues. Therefore, if a telotrisomic carrying a recessive marker on one normal chromosome and normal alleles on the other normal homologue and the telocentric is selfed, the diploid fraction of its progeny should segregate 3 : 1. All of the trisomic progeny should have normal phenotype since the normal allele will be carried by the telocentric and possibly also by one or both normal homologues except for transfer of the recessive marker to the telocentric by crossing over. If the marker is not located on the telocentric arm, normal disomic ratios should obtain also for the trisomic fraction of the progeny. Observed ratios, whether 3 : 1 : : all : 0 or 3 : 1 : : 3 : 1, can thus identify the appropriate arm for a marker. It should be noted, however, that the distinction between these two sets of ratios is possible only when the telotrisomic type can be distinguished morphologically from diploid. If the telotrisomic is not distinct or if the plants must be scored before they

are large enough to be thus classified, the ratios for the entire progeny can still be evaluated, but with less discrimination.   If half of the female gametes transmit the telocentric, the ratio among the total progeny should be 7 : 1 ; if one-third transmit it, 5 : 1.   Clearly the test is more efficient and much smaller populations are needed when the progenies can be classified into diploid and telotrisomic portions.

Three of the six tomato telotrisomics were used for such genetic tests. Four genes on chromosome 3 were tested against triplo-3L, and one gene apiece against triplo-7L and triplo-8L.   An examination of Table 2 reveals that the trisomic fractions of the progenies are immediately diagnostic of disomic or trisomic ratios ; moreover, the conclusions regarding the type of ratio are fortified by the high χ^2 values.   Thus, regarding chromosome 3, the ratios for r and wf are disomic with triplo-3L, while those for rv and sf are trisomic ; r and wf must therefore lie on 3S, rv and sf on 3L.   The

Table 2.   Segregation ratios in F_2's of different telotrisomics

Telotri-somic	Gene	Total	Progeny						
			2n			2n+1			
			Normal	Reces-sive	% Reces-sive	Normal	Reces-sive	% Reces-sive	χ^2 3:1
2n+3L	r	184	94	38	28.7	39	13	25.0	0.00
	wf	184	99	33	25.0	40	12	23.1	0.10
	rv	180	102	18	15.0	59	1	1.6	17.41
	sf	180	104	16	13.3	59	1	1.6	17.41
2n+7L	var	176*	77	31	28.7	52	15	22.3	0.22
2n+8L	cpt	131**	42	16	27.5	65	1	1.5	19.39

  *   One plant in the family was triplo-7.
  **   9 plants in the family were 2n+2(2n+8L+8L).

genes ru and sy have previously been located on 3S by means of monosomic analysis permitted by a novel compensating trisomic (Khush and Rick 1967a). The centromere must consequently lie between ru and rv.   The orientation of the linkage group, reading from the end of the short arm, must therefore be r-wf-sy-ru-centromere-rv-sf instead of the reverse order indicated on the latest linkage maps (Clayberg *et al.* 1966).   In a separate test, var of chromosome 7 gave disomic ratios with triplo-7L, hence must have its locus on 7S.

In respect to chromosome 8, three markers had previously been allocated to their respective arms by means of X-ray-induced deficiencies—l on 8S, bu and dl on 8L (Rick and Khush 1966).   Since another gene, cpt, had been mapped by standard F_2 linkage tests between l and bu, its arm location would provide critical information for delimiting the centromere.   This marker could not be efficiently located by means of induced deficiencies because it

cannot be scored in the seedling stage. It was therefore tested against triplo-8L and, as evident from the results reported in Table 2, it gave a trisomic ratio. The gene *cpt* must therefore lie on 8L, the centromere between *l* and *cpt*.

A dominant gene with dosage effects can be allocated to its respective chromosome arm simply by comparing phenotypes of heterozygous telotrisomics (as well as secondary and tertiary trisomics) with those of diploids with known dosages. Thus *La* (Lanceolate), a dominant mutant on chromosome 7, produces narrow, entire leaves with 0-2 small lateral segments when heterozygous in diploids (*La/+*). The leaves of triplo-7L with one dose of *La* were broader and usually had two pairs of lateral segments at the base of the terminal segment (Fig. 7). This departure from the typical *La/+* phenotype was in the direction expected if the *La* locus were on 7L and the heterozygotes therefore genetically *La/+/+*. The phenotype of these telotrisomics closely approximated that for *La/+/+* described by Stettler (1964).

Transmission

Systematic studies have not yet been made to determine the male transmission rates of the extra telocentric chromosomes. Of the 12 primary trisomics of the tomato, extras for only chromosomes 7, 8, and 10 are transmitted through the male, but at low rates. The telocentrics 10S and 3S are so small, however, that they can be expected to transmit as extras through pollen. That triplo-8L can be pollen transmitted is evident from the appearance of nine plants with two extra 8L telocentrics apiece in the F_2 progeny of this telotrisomic (Table 2). Taking this experience into account and also the relative arm lengths, we might expect triplo-7L to transmit through the male but not triplo-3L and triplo-4L.

Data on female transmission in $(2n+1)\times 2n$ crosses are presented in Table 3. Although the progeny sizes are not large, it is evident that the

Table 3. Transmission rates of telotrisomics in progenies of $(2n+1)\times 2n$

Telotrisomic	Total No.	2n No.	2n+1	
			No.	%
2n+3L	77	70	7	10.0
2n+4L	106	65	41	38.7
2n+7L	75	47	28	37.3
2n+8L	75	45	30	40.0
2n+3S	68	35	33	48.5
2n+10S	18	10	8	44.4

female transmission rates are quite high—higher, in fact, than those for the corresponding primary trisomics. It may be noted that the transmission rates

calculated from Tables 2 and 3 do not correspond with eath other. These discrepancies probably owe to the fact that the progenies reported in Table 2 were in heterozygous background while those in Table 3 were in the uniform background of a highly inbred line of cv VF36, to which the telotrisomics have been made isogenic by a program of repeated backcrossing. The maximum yield of telotrisomics was encountered in an F_2 of triplo-8L, in which, thanks to male transmission of extras, the number of trisomics actually exceeds that of diploids (Table 2).

Primary trisomics corresponding to the telotrisomics were rarely obtained, the only example encountered being a solitary triplo-7 yielded by the self of a triplo-7L. This rarity of related primaries is in agreement with the nonrandom segregation of members of the trivalents observed cytologically for two telotrisomics.

Discussion

Applications in linkage mapping. The present study, as well as that of Rhoades (1936), shows that telotrisomics can be effectively employed in linkage mapping. Once association between a chromosome and its linkage group has been established, it is important to determine the orientation of the linkage map and the position therein of the centromere. For this purpose it is essential to determine the arm location of two or more well-mapped markers—a task that can be accomplished with the aid of telotrisomics as demonstrated in the present study for tomato chromosome 3. Secondary and tertiary trisomics may also be used for the same purpose, but some of the telotrisomics have already proved to be more amenable and efficient because their fertility, vigor, and transmission rates are higher. For a species with 12 pairs of chromosomes, a set of 12 telotrisomics would normally be required, but for tomato, 11 would suffice because 2S acts as if totally heterochromatic. Telocentrics for the long arms of the tomato chromosomes are more useful than those for the short arms since our studies show that the former tend to be phenotypically identifiable while the latter may be difficult to identify.

Stability of telocentrics. The hypothesis of Nawashin (1916), Darlington (1939), and White (1954) that telocentric chromosomes are unstable does not seem to apply to all telocentrics of the tomato. The centromeres of three, 3L, 4L, and 8L, are not truly terminal but are flanked in each case by a heterochromatic chromomere—a situation that might explain their stability. Yet the telocentrics 7L and 10S are equally stable in spite of appearing to have terminal centromeres. The telocentric 3S with an apparently terminal centromere, on the other hand, is unstable.

As stated in the results, the centromere of telo-3S is about half the size of the normal tomato centromere, while those of telo-7L and -10S seem to be of normal size. Within the limits of resolution of the light microscope,

these results are consistent with the hypothesis that *completeness* rather than *subterminal position* determines stability of the centromere. If the chromosome is so broken that the centromere is left intact with the telocentric arm, it would be expected to function normally, but if only part of the centromere remains, it would be unstable and the telocentric subject to loss in mitoses. Several examples of entirely stable centromeres have been presented by Marks (1957) in an admirable review of the subject. He has also diagramed several kinds of breakage that can generate telocentric chromosomes with complete or incomplete centromeres. Moreover, the degree of incompleteness of the centromere can vary. The differential instability of several telocentrics of the short arm of chromosome 3B of wheat was attributed by Steinitz-Sears (1966) to the degree of completeness of the terminal centromeres.

Phenotypic effects. One of the unequivocal findings of this investigation is the markedly stronger effects on gross phenotype of the mature plant exerted by the telocentric chromosomes for the long arms than those for the short arms and the consequent close resemblance between telotrisomics for long arms and the corresponding primary trisomics. Such a relationship might be anticipated from the length of the arms *per se*, but it is our impression that the predominant influence of the long arms is out of proportion with their lengths. This indication of a pronounced genetic effect is in keeping with evidence we encountered previously in a study of tomato monosomics (Khush and Rick 1966a). We ascertained therein that nine long arms of the complement, including 3L, 4L, 7L, and 8L, represented in the present series of telotrisomics, are of such essentiality that their losses are not tolerated by the tomato sporophyte. Evidence from internal deficiencies was also presented to suggest that the same longer arms contain chromatin that per unit length is more vital for survival than that of the shorter arms.

Summary

Telotrisomics—sporophytes possessing a telocentric chromosome in addition to the normal complement—were obtained and studied for six different chromosome arms of the tomato. They originated in the progeny of the following kinds of chromosomal deviants: terminal deficiency, tertiary monosomic, and compensating trisomic. Cytological studies at meiotic pachytene established that the centromere of three telocentrics was flanked by a heterochromatic chromomere opposite the intact arm and three appeared to have terminal centromeres. Of the latter group the centromere of two appeared to be of normal size, while one was about half normal size, its chromosome being the only one of the six telocentrics to be mitotically unstable. The longer the telocentric arm, the greater the tendency to form trivalents; when trivalents are not formed, the telocentric is invariably univalent, the preferential pairing between the two normal homologues leading exclusively to 12 : 12 + telocentric anaphasic separations. Consistent with this cytological be-

havior, only one in several hundred trisomic progeny was of primary type, the rest telosomic.—The effects of the extra telocentric chromosome on gross phenotype of the plant are much greater for the long arms, and telocentrics for long arms closely resemble the corresponding primary trisomics. As a result of gametophytic elimination, the telocentric is never substituted for the corresponding normal chromosome, but can be transmitted as an extra element for all studied telocentrics on the female side and for the short arms also on the male side. The rate of female transmission generally exceeds that of the corresponding primary trisomic.—Inheritance studies of three telotriosmics permitted assignment of marker genes to their proper chromosome arms. Since efficiency of the trisomic ratio method is enhanced if the telotrisomic is phenotypically distinguishable from the diploid, trisomics for the long arms are better suited for genetic mapping purposes than those for short arms. In this fashion r and wf were proven to lie on 3S, rv and sf on 3L, var on 7S, and cpt on 8L, with correspondingly closer approximations of centromere positions. The incompletely dominant gene La was assigned to 7L by virtue of dosage effects in heterozygous triplo-7L. Considering their higher rates of transmission and less depressing effects on vigor and fertility, extra telocentrics render telotrisomics more useful for determining arm locations than either secondary or tertiary trisomics.

Literature cited

Blakeslee, A. F. and Avery, A. G. 1938. Fifteen-year breeding records of 2n+1 types in *Datura stramonium.* Cooperation in Research, Carnegie Inst., Washington Publ. 501: 315-351.

Burnham, C. R. 1962. Discussions in Cytogenetics. Burgess Publishing Company. Minneapolis. 375 p.

Clayberg, C. D., Butler, L., Kerr, E. A., Rick, C. M. and Robinson, R. W. 1966. Third list of known genes in the tomato. Jour. Hered. 57: 188-196.

Darlington, C. D. 1939. Misdivision and genetics of the centromere. J. Genet. 37: 341-364.

Endrizzi, J. E. and Kohel, R. J. 1966. Use of telosomes in mapping three chromosomes in cotton. Genetics 54: 535-550.

Goodspeed, T. H. and Avery, P. 1939. Trisomic and other types in *Nicotiana sylvestris.* Jour. Genet. 38: 381-458.

Kamanoi, M. and Jenkins, B. C. 1962. Trisomics in common rye, *Secale cereale* L. Seiken Zihô 13: 118-123.

Khush, G. S. and Rick, C. M. 1966a. The origin, identification and cytogenetic behavior of tomato monosomics. Chromosoma 18: 407-420.

— and — 1966b. The use of tertiary trisomics in linkage mapping. Genetics 54: 343.

— and — 1967a. Novel compensating trisomics of the tomato: cytogenetics, monosomic analysis, and other applications. Genetics 55: (In press).

— and — 1967b. Haplo-triplo-disomics of the tomato: origin, cytogenetics, and utilization as a source of secondary trisomics. Biol. Zbl. 86: (In press).

— and — 1967c. Studies on the linkage map of chromosome 4 of the tomato and on the transmission of induced deficiencies. Genetica 38: (In press).

Marks, G. E. 1957. Telocentric chromosomes. Amer. Nat. XCI: 223-232.

McGinnis, R. C., Andrews, G. Y. and McKenzie, R. I. H. 1963. Determination of chromosome arm carrying a gene for chlorophyll production in *Avena sativa.* Can. J. Genet.

Cytol. 5: 57-59.

Moseman, H. G. and Smith, L. 1954. Gene location by three point test and telocentric half chromosome fragment in *Triticum monococcum*. Agron. J. 46: 120-124.

Nawashin, S. G. 1966. Sur quelques indices de l'organisation interne des chromosomes. Timijaseffs. Festschrift: 185-274.

Rhoades, M. M. 1936. A cytogenetical study of a chromosome fragment in maize. Genetics 21: 491-502.

Rick, C. M. and Khush, G. S. 1966. Chromosome engineering in *Lycopersicon*. *In*: Chromosome Manipulations and Plant Genetics. R. Riley and K. R. Lewis Editors. A supplement to Heredity 20: 8-20.

Sears, E. R. 1954. The aneuploids of common wheat. Univ. Missouri Res. Bull. 572: 1-58.

— 1962. The use of telocentric chromosomes in linkage mapping. Genetics 47: 983.

Steinitz-Sears, L. M. 1966. Somatic instability of telocentric chromosomes in wheat and the nature of the centromere. Genetics: 54: 241-248.

Stettler, R. F. 1964. Dosage effects of the Lanceolate gene in tomato. Amer. Jour. Bot. 51: 253-264.

Tsuchiya, T. 1960. Cytogenetic studies of trisomics in barley. Jap. Jour. Bot. 17: 177-213.

White, M. J. D. 1954. Animal Cytology and Evolution. 2nd edition. Cambridge University Press. Cambridge. 454 p.

White, T. G. and Endrizzi, J. E. 1965. Tests for the association of marker loci with chromosomes in *Gossypium hirsutum* by the use of aneuploids. Genetics 51: 605-612.