Two-Dimensional Spreads of Synaptonemal Complexes from Solanaceous Plants. VI. High-Resolution Recombination Nodule Map for Tomato (*Lycopersicon esculentum*)

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ABSTRACT

We have produced a high-resolution physical recombination map for tomato chromosomes by determining the frequency and distribution of recombination nodules (RNs) on tomato synaptonemal complexes (SCs). We present evidence that there is a 1:1 relationship between RNs and chiasmata. Every SC has at least one RN. There are no RNs at the ends of SCs, in kinetochores, or in the heterochromatic short arm of SC *2* that carries the nucleolus organizer. RNs are more common per unit length of SC in euchromatin compared with SC in heterochromatin. The average number of RNs per SC and the average number of RNs per SC arm are directly correlated with the length of SC in euchromatin. When SCs have only one RN, that RN occurs on the long arm more frequently than predicted based on SC arm length. Patterns of multiple RNs on SCs indicate RN (crossover) interference. RNs probably can occur anywhere on SCs in euchromatin, but RNs are not distributed randomly along SCs in euchromatin or in heterochromatin. The lengths of tomato's physical recombination (RN) map, classical genetic linkage map, and molecular linkage map all differ from each other for a variety of reasons.

LINKAGE maps for classical genetic markers, isozymes, restriction fragment length polymorphisms (RFLPs), and randomly amplified polymorphic DNAs (RAPDs) indicate the linear order of markers as well as the recombination rate between markers (O'BRIEN 1990). However, linkage maps are of limited use in physically positioning markers on chromosomes because recombination is not evenly distributed along chromosomes. For instance, crossing over is uncommon in heterochromatin and sometimes uncommon near centromeres (*e.g.,* MATHER 1939; YAMAMOTO and MIKLOS 1978; for reviews, see BROWN 1966; COMINGS 1972; RESNICK 1987). In contrast, crossing over is more common in euchromatin, especially near telomeres, and sometimes more common near centromeres and euchromatin/heterochromatin borders (*e.g.,* LEVAN 1935; LINNERT 1955; JONES 1978; FLETCHER and HEWITT 1980; DE LA TORRE *et al.* 1986).

One way to use linkage maps for assigning physical locations to markers is to use *in situ* hybridization to place a few markers at physical sites on chromosomes (*e.g.,* LAWRENCE *et al.* 1990) and interpolate the position of enclosed linkage markers. However, this method is limited by the resolution of *in situ* hybridization and the assumption that linkage map distances are proportional to physical distances between *in situ* markers. Alternatively, a linkage map could be used to assign physical positions to markers if there were complementary physical maps of recombination. Such physical maps of recombination also would be useful for investigating the relationship between chromosome structure and crossing over.

Because a chiasma forms as a result of crossing over (TEASE 1978; for a review, see WHITEHOUSE 1969), descriptions of the distribution and frequency of chiasmata on chromosomes are physical maps of recombination. However, there are a number of problems with using chiasma maps as crossover maps. For example, (1) During stages when chiasmata are visible, it is often difficult to relate contracted bivalents to specific chromosomes or linkage groups. (2) On small chromosomes it is difficult to count and position chiasmata, and even on large chromosomes, chiasmata that are close together cannot be resolved (*e.g.,* STACK *et al.* 1989). (3) Bivalents at diakinesis through metaphase I are usually so contracted that observations of the positions of chiasmata are imprecise. (4) At diplotene, when the chromosomes are longer, twists are often indistinguishable from chiasmata. (5) There is disagreement over whether a chiasma can move from the original site of the crossover event, *i.e.,* terminalize (JONES 1977; LOIDL 1979; MAGUIRE 1979). (6) Species for which we have linkage maps have short chromosomes on which chiasmata are difficult to position and count, while species that have long chromosomes and the potential for good chiasma maps lack linkage maps (*e.g.,* grasshoppers; RUFAS *et al.* 1987). As a result of one or more of these problems, physical maps of crossing over based on frequency and distribution of chiasmata are not easily compared to linkage maps.

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Structures called recombination nodules (RNs) also have been related to sites of crossing over. RNs are spherical to ellipsoidal structures ~100 nm in their longest dimension that lie on the central region of synaptonemal complexes (SCs) during mid- to late pachynema (see CARPENTER 1975 for a review with many subsequent reports). Because RNs are generally correlated with crossing over and chiasmata, RNs are thought to lie at sites of crossover events and future chiasmata (*e.g.*, CARPENTER 1975, 1979, 1988; HOLM and RASMUSSEN 1980; ALBINI and JONES 1988; STACK *et al.* 1989; HERICKHOFF *et al.* 1993). Given this relationship, descriptions of RN distribution and frequency on SCs are physical recombination maps that avoid many problems of chiasma maps. For example, (1) In some organisms, individual SCs have been related to specific chromosomes and linkage groups (SHERMAN and STACK 1992; JONES and DE AZKUE 1993). (2) RNs are easy to count even on the shortest SCs (*e.g.*, see RAHN and SOLARI 1986), and there is no problem resolving nodules, even if they lie immediately adjacent to each other (STACK and ANDERSON 1986a). (3) Measurements of the positions of RNs along SCs can be more precise, because RNs are smaller than chiasmata (~0.1 *vs.* ~1.0 μm) and because RNs are visible in pachynema when chromosomes are five to 10 times longer than in diakinesis when chiasmata are visible (STACK 1984; STACK *et al.* 1989). (4) So far there is no evidence that RNs move from their initial positions, *i.e.*, there are no reports of RNs terminalizing. (5) Because SCs and RNs probably occur in all eukaryotes that have normal meiosis, RNs could be used to produce physical maps of recombination for most eukaryotes, including those for which linkage maps are available.

In spite of the advantages of RN maps as physical maps of recombination, there are reasons why detailed RN maps have not been reported; they are as follows: (1) In the past, when SCs were studied primarily by three dimensional reconstructions of pachytene nuclei, it was difficult to reconstruct enough nuclei to obtain detailed maps of RNs. (2) As techniques for spreading whole sets of SCs were introduced (COUNCE and MEYER 1973; GILLIES 1981; STACK 1982; ALBINI *et al.* 1984; HOLM 1986), it became practical to analyze more sets of SCs. However, SCs were usually stained with silver for high contrast, and the most commonly used protocols for silver staining do not reveal RNs (SHERMAN *et al.* 1992). Other stains such as phosphotungstic acid (PTA) and uranyl acetate-lead citrate (UP) reveal RNs (*e.g.*, STACK and ANDERSON 1986a), but these are general stains of lower contrast so RNs are often obscured by chromatin. (3) Some organisms do not have enough RNs visible at any one time to account for all the crossing over and chiasmata observed subsequently (*e.g.*, RASMUSSEN and HOLM 1978; GOLDSTEIN and TRIANTAPHYLLOU 1981; GILLIES 1983a; KEHLHOFFNER and DIETRICH 1983). (4) In many cases it has not been possible to differentiate all the SCs in a set, much less relate each of them to a specific chromosome (*e.g.*, RAHN and SOLARI 1986; ALBINI and JONES 1988; BOJKO 1989).

We selected tomato (*Lycopersicon esculentum*) for an investigation of the frequency and distribution of RNs on SCs because tomato has none of the disadvantages listed above. For example, we can prepare large numbers of complete SC sets on which RNs can be observed after staining with uranyl acetate-lead citrate (UP) or a new silver staining technique (SHERMAN *et al.* 1992). The number and distribution of RNs in mid- and late pachynema correspond closely to the number of crossovers and the pattern of chiasmata observed during diakinesis (STACK and ANDERSON 1986b; HERICKHOFF *et al.* 1993). Every tomato SC is identifiable and related to a specific chromosome and linkage group (SHERMAN and STACK 1992). In addition, tomato has among the best linkage maps available (TANKSLEY *et al.* 1992) for comparison with a physical map of recombination.

Here we describe a RN map for tomato SCs and compare the data with the location and frequency of chiasmata and with genetic and molecular linkage maps. In addition, we examined the location of RNs in relation to each other (interference) and to chromosome structure (euchromatin, heterochromatin, kinetochores, and telomeres).

MATERIALS AND METHODS

Diploid tomato (*L. esculentum* var. cherry) seeds were planted monthly and grown to maturity in a greenhouse where the temperature was maintained at 20–25°C. Anthers containing microsporocytes in pachynema were removed from plants that were 2–3 months old. Plants older than 3 months were not used to prepare SC spreads. Although we attempted to spread SCs throughout the year, no useable spreads were produced during the months of November or December (even though lights on a 12-hr regime were used during the winter to increase the effective day length).

Two-dimensional spreads of SCs were produced by hypotonically bursting primary microsporocytes on Falcon plastic-coated glass slides as described by SHERMAN *et al.* (1992). SC spreads were fixed with paraformaldehyde and stained with either silver nitrate (Ag) according to SHERMAN *et al.* (1992) or with uranyl acetate and lead citrate (UP) according to STACK and ANDERSON (1986b). SC sets were located by phase contrast light microscopy, picked up on grids, and examined with an AEI 801 electron microscope. Analyzable sets were photographed, and SCs were traced onto acetate film from prints. The positions of the kinetochores and euchromatin/heterochromatin borders were marked (SHERMAN and STACK 1992). The kinetochores generally appeared as stained fibrous masses ~1 μm in diameter on the SCs (Figures 1–11 and 13). Because SC in euchromatin stains more densely than SC in heterochromatin, the euchromatin/heterochromatin borders were identified as segments of each SC where staining density changes (Figures 1–11 and 13). The positions of RNs, kinetochores, and euchromatin/heterochromatin borders were marked on each SC. The SCs were then measured utilizing a Hewlett Packard graphics tablet and a computer program written for measuring SCs.

Only SC sets in middle to late pachynema as defined by STACK and ANDERSON (1986a,b) were used to generate the RN map. Each SC was identified according to its relative length, arm ratio, and percent heterochromatin (SHERMAN

and STACK 1992). Most measurements came from complete sets of SCs. Incomplete sets of SCs were also measured if every SC in the group could be unambiguously identified. RN locations were determined for >400 of each of tomato's 12 SCs. (Compare Figures 7, 10 and 11 with Figure 13 to observe different RN positions on the same SCs.) Total SC lengths varied from set to set, but the relative length of SCs and SC arms, *i.e.*, arm ratios, within each set remained consistent (SHERMAN and STACK 1992). For comparison of RN positions from SC set to set, RN positions were measured in micrometers from the centromere, and then RN positions were described as a percentage of the long or short arm measured from the centromere. Using the average lengths for each of the 12 SCs and their average arm ratios, each of the SCs was divided into 0.1-μm segments. Divisions of this size were selected because they are the same length as a RN. Each observed RN was placed in one of these 0.1-μm divisions based on its position as a percentage of the arm measured from the centromere. The combined data for each SC is shown in a series of cumulative histograms (Figure 12 and see APPENDIX).

RN distributions on each SC were converted into map units by first dividing the number of observed RNs (crossovers) in each 0.1-μm segment by the number of SCs examined to yield a quotient (the average number of RNs in the segment) that was then multiplied by 50 map units (number of map units per crossover) to give a product that is the number of map units in the 0.1-μm segment. For instance, if three RNs were observed in a particular 0.1-μm segment of SC *4* and 430 SCs *4* were measured, this 0.1-μm segment would be [(3 ÷ 430) × 50] 0.35 map units in length.

To position map units on diagrams of SCs (Figure 18), the map lengths of 0.1-μm segments were added moving from the centromere toward the telomere in an arm. When the map unit sum reached or exceeded an integer (usually 1), a perpendicular tick was made at that site on the diagram. In a few cases there were enough nodules in one 0.1-μm segment that two map units were assigned to a single segment. In these cases, two ticks were placed within the segment. Any fraction of a map unit over the integer was carried forward as the map units in successive segments were summed until another integer was reached and another tick was added. This process was repeated until the end of the arm was reached. After ticks were positioned on one SC arm, the same procedure was used to position ticks on the other arm.

To test one aspect of random distribution of RNs in euchromatin, we compared the observed numbers of 0.1-μm segments in long arms of each SC carrying 0, 1, 2, 3, *etc.*, RNs with the expected number of segments with the same numbers of RNs based on normal curves. For this we used the data sets in the APPENDIX (also see the cumulative histograms illustrated in Figure 12) and calculated the expected normal curves based on the means and standard deviations of the data sets using Microsoft Excel 4.0 (Formula-Paste Function-Statistical-NORMDIST). These normal distributions were compared with the observed data sets using chi-squared tests in Microsoft Excel 4.0 (Formula-Paste Function-Statistical-CHITEST).

To see if the position of one RN in the euchromatin of an arm influences the position of another RN in the euchromatin of the same arm (interference), we measured the distances between RNs when only two RNs were found in one SC arm. Such RN pairs were grouped into intervals based on the fraction of euchromatic arm length between them (*e.g.*, 0–0.1, 0.1–0.2, *etc.*). Assuming random and independent placement of each nodule on a segment of SC, the expected number of RN pairs separated by each interval was calculated as the product of the total number of observed pairs in all intervals multiplied by the theoretical probability that a pair of nodules will fall in that particular interval. The theoretical probability of RN pairs with a separation anywhere from 0 to the maximum value (d) for an interval was determined using the formula $1 - (1 - d)^2$. The probability that any pair of RNs will be separated by a distance falling in a particular separation interval is equal to the probability of the two RNs being separated by d or less minus the probability of the two RNs being separated by the next smaller d value or less (Table 1). For example, the probability of a RN pair being separated by 0.2–0.3 of the SC arm length in euchromatin is $1 - (1 - 0.3)^2 - [1 - (1 - 0.2)^2] = 0.15$. Note that (0.19 + 0.17 + 0.15 = 0.51) half of the RN pairs are expected to be separated by ≤0.3 of the SC length in euchromatin, so the expected mean separation of RN pairs is $\sim 1/3$ of the SC length in euchromatin. The observed and expected frequencies of RN pairs in the different separation intervals were plotted for each SC separately (data not shown), and the data from all 12 SCs were combined and plotted as well (Figure 17).

TABLE 1

Predicted distribution of intervals between RN pairs

Interval between two RNs	Expected frequency of RN pairs/interval
0–0.1	0.19
0.1–0.2	0.17
0.2–0.3	0.15
0.3–0.4	0.13
0.4–0.5	0.11
0.5–0.6	0.09
0.6–0.7	0.07
0.7–0.8	0.05
0.8–0.9	0.03
0.9–1.0	0.01

In this table, we limited consideration to cases where there are two RNs per SC arm. In addition, we assumed that each RN is located independently and randomly on the SC arm. For each interval, the maximal value (d) was used to calculate the expected frequency using the formula $1 - (1 - d)^2 - \{1 - [1 - (d - 0.1)]^2\}$. Intervals are expressed as a fraction of SC arm length.

RESULTS AND DISCUSSION

Variables in preparing spreads of SCs have no discernable effect on the frequency and placement of RNs: Recombination has been reported to be affected by several factors, including temperature (WILSON 1959; MAGUIRE 1968; GAVRILENKO 1984; LOIDL 1989), age of the plant (GRIFFING and LANGRIDGE 1963) and genetic background (*e.g.*, HOLM and WANG 1988; CORNU *et al.* 1989). To decrease possible variation in recombination between experiments, we maintained our plants in a controlled-temperature greenhouse, used plants that were from 2 to 3 months old, and used seed from plants that had been inbred for more than five generations.

RN maps for each of the SCs were prepared as described in the MATERIALS AND METHODS. Data for these maps were gathered from SC spreads obtained from 30 different tomato plants, prepared in different years and at different times of the year, and stained in two different ways. In addition, spreads of SCs differed in total

TABLE 2

Comparison of staining methods, numbers of RNs per SC set, and lengths of SC sets

Stage, stain	No. of sets observed	Mean no. of RNs	Mean length of SC sets (μm)
Middle pachynema, silver (Ag) staining	135	21.1 ± 3.0	212.3 ± 30.8
Late pachynema, silver (Ag) staining	65	21.3 ± 3.0	208.0 ± 27.2
Middle pachynema, uranyl acetate/ lead citrate (UP) staining	23	20.9 ± 3.8	209.0 ± 31.6
Late pachynema, uranyl acetate/ lead citrate (UP) staining	47	21.8 ± 4.1	213.0 ± 19.9

length, substage of pachynema, and apparent proportion of heterochromatin in each SC arm. While it is conceivable that any or all of these factors might contribute to variations in RN frequency and position between sets, we believe pooling the data for each SC is justified because when the frequencies of RNs per set were compared between seven individual plants, there were no significant differences (t-test, $P > 0.2$). When SCs were grouped according to the season in which they were spread, RN frequencies per set were not significantly different in the winter, spring, summer, or fall (t-test, $P > 0.8$). Comparing silver-stained SC/RNs to UP-stained SC/RNs, there was no significant difference between the mean number of RNs (t-test, $P = 0.68$) or the apparent distribution of RNs (Table 2, and see SHERMAN *et al.* 1992). There was no significant difference between the mean numbers of RNs per SC set in middle *vs.* late pachynema (t-test, $P = 0.68$) or the mean length of SC sets (t-test, $P = 0.34$) whether stained with silver or uranyl acetate-lead citrate (UP) (Table 2; and see SHERMAN *et al.* 1992). There is some variation in total length of individual sets of SCs (207.4 ± 25.7 μm, mean ± SD; SHERMAN and STACK 1992), but relative lengths and arm ratios of SCs within sets remain constant (<1% variation, SHERMAN and STACK 1992) so relative positions of RNs could be compared from SC to SC.

There is a 1:1 relationship between recombination nodules (RNs) and chiasmata: Tomato chromosomes are small, and chiasmata at diakinesis-metaphase I are difficult to count. Because each chromosome has a large block of pericentric heterochromatin where chiasmata usually are not found, one or more chiasmata in only one arm will result in a rod bivalent, and one or more chiasmata in both arms will result in a ring bivalent. On this basis (in spite of the tendency to under estimate the number of chiasmata), it is routine in tomato cytogenetics to count rod bivalents as having one chiasma and ring bivalents as having two chiasmata. Counting chiasmata this way, we analyzed 147 complete sets of chromosomes at diakinesis and found an average of 17.1 ± 2.0 chiasmata per set, *i.e.*, an average of seven rods and five rings. When we counted the number of RNs in 278 complete sets of mid- to late pachytene SCs, the average number of RNs was 21.25 ± 3.00. The average number of RNs per set was even higher (21.89) when it was calculated from the average number of RNs per SC (Table 4—where more observations were possible because incomplete sets were used). In either case, the average number of RNs observed is not in close agreement with the average number of chiasmata determined by counting rod and ring bivalents (21 or 22 *vs.* 17). However, because the method used to count chiasmata ignores the possibility that more than one chiasma can occur in one arm, we predicted the frequency of rod and ring bivalents on the assumption that each RN will form a chiasma. By this method, a rod bivalent will result from one or more RNs in only one arm of a SC, while a ring bivalent will result from one or more RNs in both arms of a SC (Table 3). When the observed and predicted frequency of rod or ring bivalents are compared using the Kolmogorov-Smirnov goodness of fit test, the observed and predicted frequencies are statistically indistinguishable (dMax = 4, $K = 9$, $n = 100$, $\alpha > 0.5$) (ZAR 1984). Thus, it appears that a chiasma forms at the site of each RN. This conclusion is consistent with observations in humans (RASMUSSEN and HOLM 1978), *Sordaria humana* (ZICKLER and SAGE 1981), *Allium fistulosum* (AL-

TABLE 3

Observed *vs.* predicted percentages of bivalent sets at diakinesis containing 0–12 rods

No. of rods	Percentage of sets	
	Observed chiasmata[a]	Predicted chiasmata[b]
0	0	0
1	0	0
2	1	0.3
3	5	5
4	7	8
5	12	10
6	14	15
7	20	21
8	18	14
9	14	13
10	6	11
11	3	4
12	0	0

[a] There were 147 sets of chromosomes in diakinesis analyzed.

[b] The predicted percentages were determined from observed distributions of RNs at mid- to late pachynema assuming that one or more RNs in one arm of a SC will result in a rod bivalent at diakinesis, while one or more RNs in both arms of a SC will result in a ring bivalent. There were 278 pachytene sets of SCs analyzed.

TABLE 4

Number of RNs on each SC

SC no.	Mean length	No. of SCs observed	Mean no. of RNs per SC	Observed no. of SCs with 1 RN	2 RNS	3 RNs	4 RNs
1	30.0	457	2.48	70	196	165	26
2	21.3	453	2.08	161	245	43	4
3	23.1	438	2.10	114	246	67	11
4	20.8	430	1.89	137	247	38	8
5	16.2	419	1.67	224	173	19	3
6	18.5	444	1.73	202	207	32	3
7	18.5	445	1.77	181	218	42	4
8	18.5	456	1.68	207	210	38	1
9	16.2	424	1.58	200	203	20	1
10	16.2	422	1.66	193	202	27	0
11	16.2	422	1.66	194	206	20	2
12	14.0	418	1.59	190	194	34	0
Total	229.5	5228	21.89	2073	2547	545	64

BINI and JONES 1988), *Locusta migratoria* and *Chloealtis conspersa* (BERNELOT-MOENS and MOENS 1986), and tomato translocation heterozygotes (HERICKHOFF *et al.* 1993), as well as with the developmental sequence showing the conversion of RNs to chiasmata in *Bombyx mori* (HOLM and RASMUSSEN 1980).

Every SC has at least one RN: On average there are ~22 RNs per set of SCs (Table 4), and the average number of RNs per SC ranges from 1.6 to 2.5 (Table 4). If each RN has an equal chance of falling on any of the 12 SCs in a set and the presence of one or more RNs on a SC has no influence on that SC acquiring more RNs, *i.e.*, RNs occur at random, the probability that a particular SC in a set will have no RNs is $[(11/12)^{22}]$ 0.15. However, every SC was observed to have one or more RNs (Table 4, Figures 1–11, 13), so RNs must not be distributed randomly on SCs (for further discussion, see HOLM 1987; CARPENTER 1988). The presence of least one RN on every tomato SC is consistent with the observation that univalents have not been observed in normal diploid tomato (CHARLES RICK, personal communication).

There are no RNs at the ends of SCs: Tomato SCs and pachytene chromosomes terminate in telomeres that stain darkly with silver or UP (Figures 1–11, 13). For 23 of 24 SC ends (omitting the heterochromatic short arm of SC *2*), RNs closest to telomeres ranged from 0.1 to 0.6 μm (average = 0.4 ± 0.1 μm) away from the ends (Fig. 12 and see APPENDIX). There are no other comparable segments of SC in euchromatin that consistently lack RNs.

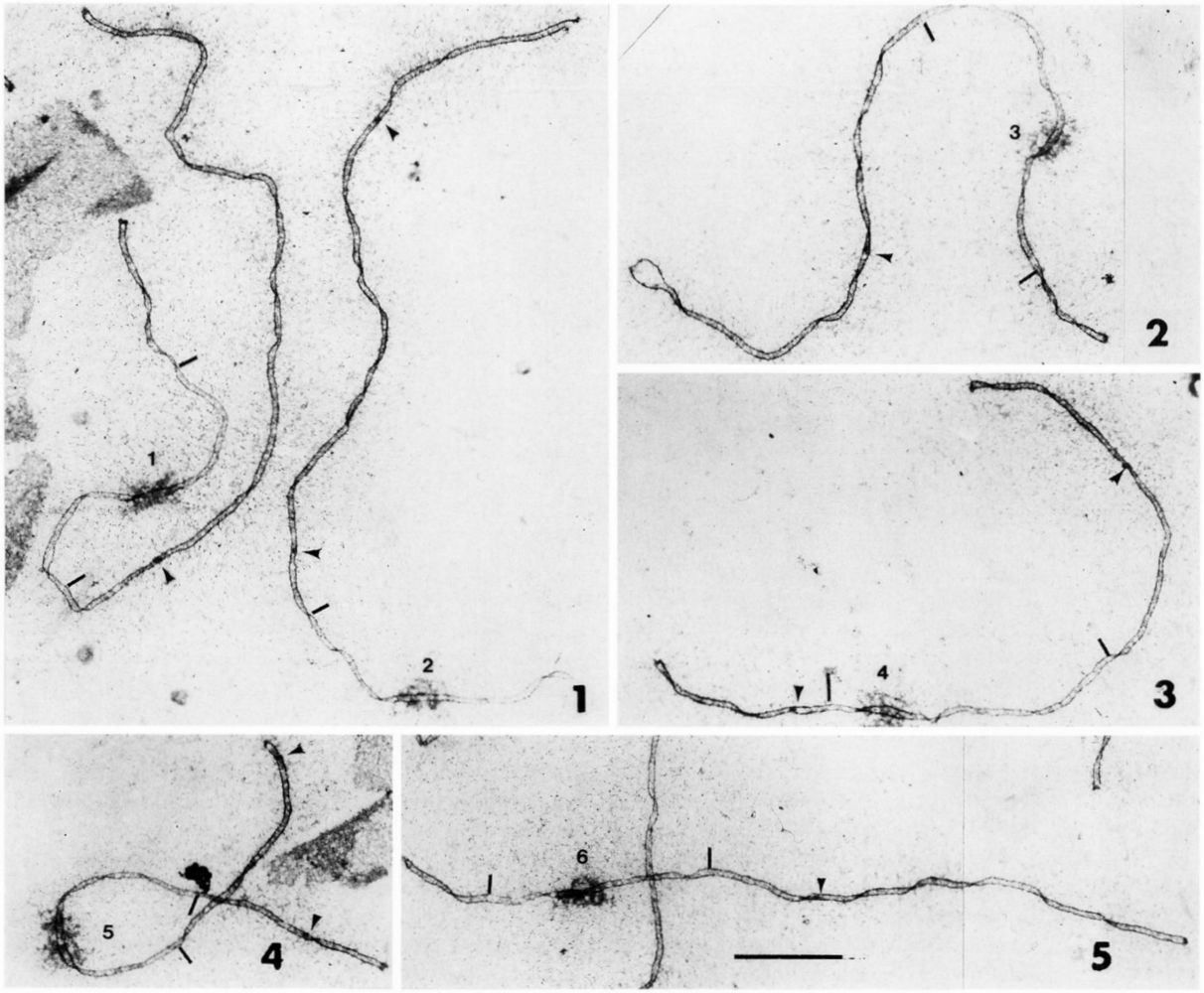
The lack of RNs (crossing over) close to telomeres may be related to the presence of telomeric and subtelomeric repeated DNA sequences on tomato chromosomes (see LAPITAN 1992 for a review). The telomeric repeat consists of 30–60 kb, while the TGRI satellite repeat consists of 25–1000 kb. These two sequences are separated by >150 kb of uncharacterized DNA. Because crossing over can occur in the TGRI satellite (GANAL *et al.* 1992), suppression of crossing over must involve the telomeric sequences and possibly the uncharacterized subtelomeric DNA. In this regard, TANKSLEY *et al.* (1992) reported clustering of molecular markers near telomeres on some tomato chromosomes and suggested that these markers probably appear clustered in linkage maps due to low levels of recombination near the ends of some tomato chromosomes.

There are no reports of RNs at telomeres in other species either, so this lack of crossing over may be important for protecting telomeric repeats from unequal crossing over. On the other hand, ASHLEY *et al.* (1993) have evidence that telomeric repeats promote recombination in *Mus domesticus*.

While there are no reports of RNs very near telomeres, there are reports of RNs concentrated toward the telomeres of *S. humana, Coprinus cinereus,* and humans (HOLM *et al.* 1981; ZICKLER and SAGE 1981; SOLARI 1982). However, tomato RNs are neither concentrated nor scarce towards telomeres. On average at ~0.6 ± 0.14 μm (±SD) proximal to telomeres, the frequency of RNs is similar to the rest of SC in euchromatin.

There are no RNs in kinetochores: From midthrough late pachynema in tomato, kinetochores are prominent, roughly spherical, darkly staining structures that are ~1 μm in diameter. Heterochromatin occurs to either side of kinetochores in every SC. Lateral elements in euchromatin and in kinetochores stain more densely than lateral elements in heterochromatin (Figures 1–11) (STACK and ANDERSON 1986a,b). However, in spite of the similarity in staining of SCs in euchromatin and in kinetochores, RNs were never observed associated with SC in kinetochores. RNs are very rare even close to kinetochores. For example, we observed only seven out of 9562 RNs within 10% of the SC length from kinetochores.

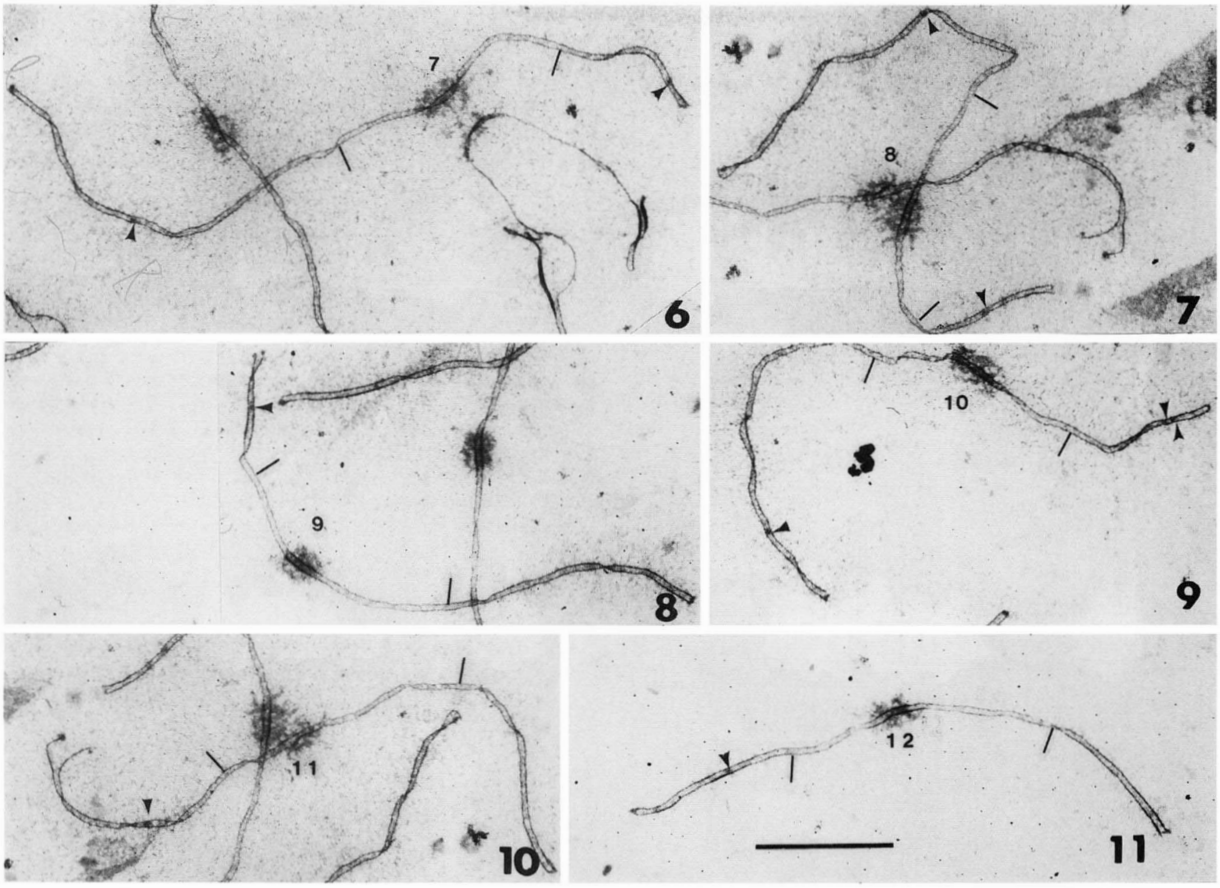
Similarly, LESLEY (1937), BROWN (1949), and BARTON



FIGURES 1–11.—An idiogram of silver stained tomato SCs. Each SC is numbered at its kinetochore. Arrowheads indicate RNs, and lines indicate euchromatin/heterochromatin borders. Bar, 2 μm.

(1951) observed no chiasmata near tomato centromeres. In many organisms, crossing over and gene conversion do not seem to occur near centromeres (see RESNICK 1987 for a review), and a number of investigators have specifically noted the absence of RNs in and near kinetochores in a variety of species (*e.g., Schizophyllum commune,* CARMI *et al.* 1978; humans, SOLARI 1980; *Zea mays,* GILLIES 1983b; *B. mori,* RASMUSSEN and HOLM 1984; *C. cinereus,* HOLM 1987; *Gallus domesticus,* RAHN and SOLARI 1986; *M. domesticus,* GLAMANN 1986; *Neurospora crassa,* BOJKO 1989; but, for exceptional cases where RNs are regularly observed near kinetochores of *A. fistulosum* and *A. porrum* SCs, see ALBINI and JONES 1988; STACK 1993). Thus, it appears that RNs and crossing over are excluded from kinetochores and usually inhibited near kinetochores. Exclusion of crossing over from kinetochores may be important for protecting kinetochore repeat sequences (WILLARD 1990) from unequal crossing over that could result in impaired kinetochore function.

RNs are more common in euchromatin than in heterochromatin: Although RNs were observed in pericentric heterochromatin of every SC arm except the short arm of SC *2* (Figures 1, 12, 13), RNs are generally 20–50 times more frequent per unit length of SC in euchromatin than in heterochromatin (Table 5). The number of RNs on SCs in heterochromatin appears somewhat greater in the histograms (Figure 12) and APPENDIX than was actually observed (Table 6). This is because the SCs below the histograms illustrate the average location of euchromatin/heterochromatin borders for each SC, while the observed locations of these borders vary from set to set (Table 2 in SHERMAN and STACK 1992; see standard deviations bars on diagrammatic SCs in Figure 12). As a result, some individual segments of SC with RNs in euchromatin fall on the heterochromatin side of the diagrammatic SCs. There are a number of possible explanations for the observed variation in location of the euchromatin/heterochromatin borders. (1) There may be a difference in packaging euchromatin



FIGURES 1–11.—*Continued*

and heterochromatin from cell to cell, *i.e.*, the position of the border may vary, perhaps like that observed in position effect variegation (DEMEREC 1940). (2) While any noticeably stretched SC was not included in our study, slight stretching of individual SCs would result in moving the border. (3) The transition of euchromatin to heterochromatin is gradual, so the point indicated may not be the actual border. Regardless of the cause(s) of the variation, we believe the numbers of RNs in heterochromatin determined from individual observations are more accurate than the numbers counted on the histograms.

The short arm of chromosome *2* is heterochromatic and carries the nucleolus organizer region (NOR) (MOENS 1968). While this arm is often broken or desynapsed, we never observed RNs in synapsed parts of this arm (Figures 1 and 12). Similarly, LESLEY (1937) and BROWN (1949) observed no chiasmata in the short arm of chromosome *2*, and BARTON (1951) established that there is no crossing over in this region by observing heteromorphic satellites. Because at least a few RNs were observed in pericentric heterochromatin of all other arms, including that in the long arm of chromosome *2*, the lack of RNs in the short arm of chromosome *2* may be related to its association with the NOR. This observation is similar to other reports of no crossing over in NORs (NATARAJAN and GROPP 1971; GOSALVEZ *et al.* 1986). However, NORs are usually associated with heterochromatin, so it is difficult to distinguish the influence of heterochromatin from the influence of NORs on crossing over.

Our observations are consistent with numerous earlier observations that indicate a lack of RNs and crossing over in heterochromatin in a variety of organisms (for reviews, see JOHN and LEWIS 1965; BROWN 1966; COMINGS 1972; YUNIS and YASMINEH 1972; LOIDL 1987). There are several possible explanations for reduced crossing over in heterochromatin. (1) The compaction of heterochromatin prevents penetration by RNs and/or components of RNs (STACK 1984). (2) Late synapsis of heterochromatin might inhibit RN formation (STACK and ANDERSON 1986a,b). (3) The synapsis of heterochromatin might be imperfect so recombination is inhibited (see LOIDL 1987 for review).

By superimposing linkage maps on tomato pachytene chromosomes, TANKSLEY *et al.* (1992) showed clustering of molecular markers near centromeres on some tomato chromosomes. However, this appearance may be due to the pattern and frequency of crossing over rather than the physical proximity of these markers to centro-

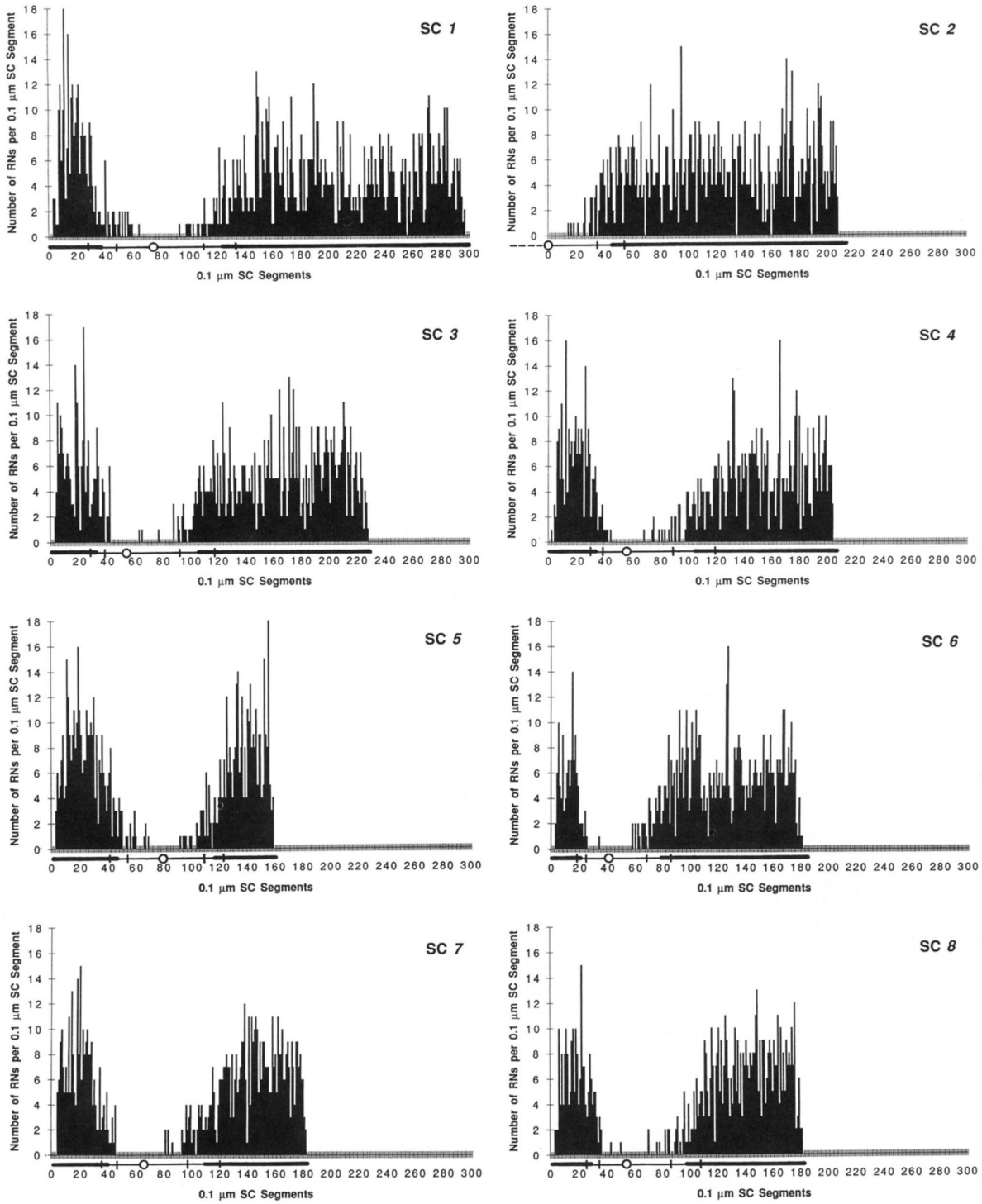


FIGURE 12.—Cumulative histograms showing the distribution of RNs on tomato SCs *1–12*. These histograms are based on the data sets presented in the APPENDIX. Abscissas represent each SC divided into 0.1-μm segments. The height of each bar represents the number of RNs observed in a particular segment as read on the ordinate scale. Under each abscissa an average SC is diagrammed in proper alignment to the histogram above. In these diagrammatic SCs, thick horizontal lines indicate SC in euchromatin, thin horizontal lines indicate SC in heterochromatin, open circles indicate kinetochores, and vertical (perpendicular) lines to either side of euchromatin/heterochromatin borders indicate one standard deviation in the observed location of the border from SC to SC. Note that there are no RNs at the ends of SCs or in kinetochores.

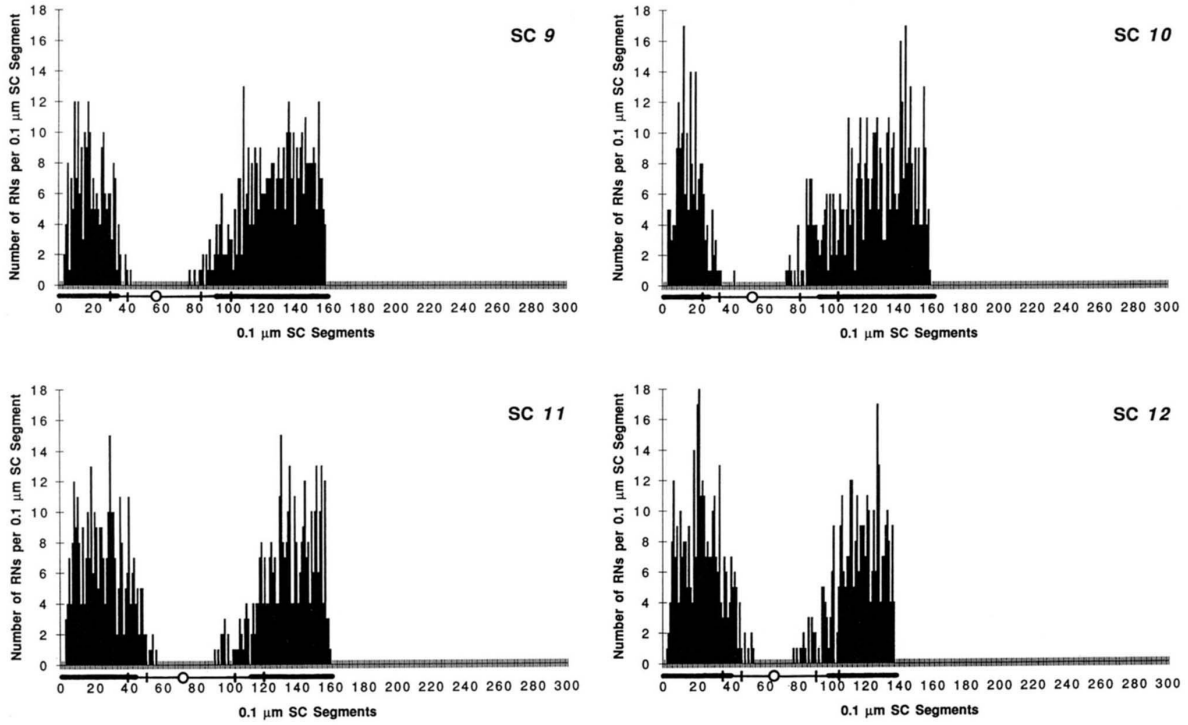


FIGURE 12.—*Continued.* RNs are relatively rare in heterochromatin except near euchromatin/heterochromatin borders. Bar, 10 μm.

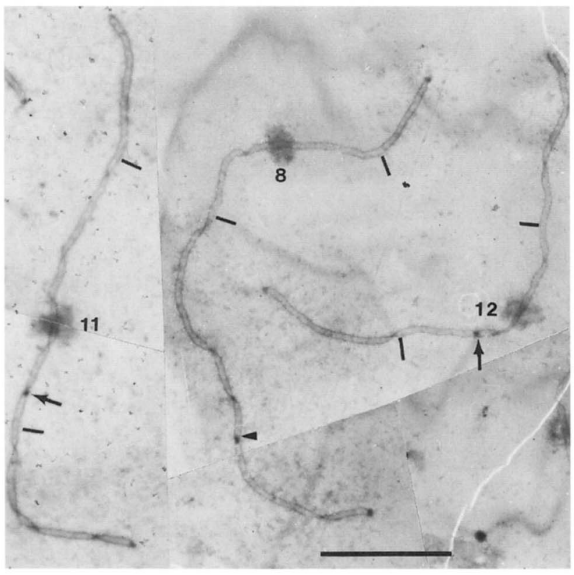


FIGURE 13.—Three uranyl acetate and lead citrate (UP)-stained SCs numbered ar the kinetochores. SCs *11* and *12* are relatively rare in having RNs (arrows) in heterochromatin, while SC *8* is more typical in having a RN in euchromatin (arrowhead). Lines indicate euchromatin/heterochromatin borders. Note that the positions of RNs on each of these SCs is different from the positions of RNs on the corresponding chromosomes shown in Figures 7, 10, and 11. Bar, 2 μm.

meres or each other. In other words, SC in pericentric heterochromatin and euchromatin/heterochromatin borders represent large physical distances on chromosomes where there is reduced crossing over. Because of this, tightly linked molecular markers in and near heterochromatin may appear to be clustered due to low levels of recombination when these markers are actually physically distant from one another.

RN numbers are related to SC length: When the mean (or relative) length of each SC (with the exception of SC *2* because we do not know its total length accurately) is plotted against the average number of RNs on each SC, the r^2 value for a regression line through these eleven points is 0.96 ($y = 0.06x + 0.67$, $P < 0.0001$; Table 4, Figure 14), indicating that 96% of the variability in average numbers of RNs per SC is related to average SC length. Similarly, RASMUSSEN and HOLM (1978) found a strong correlation between the length of human SCs and the number of RNs they carry ($r^2 = 0.92$), and RAHN and SOLARI (1986) found a linear relationship between SC length and RN numbers for the longest chromosomes in *G. domesticus*. These observations are consistent with MATHER's (1938) observations in *Stenobrothrus parallelus, Yucca flaccida,* and *L. migratoria* of a linear relationship between the length of the longer chromosomes and the number of chiasmata they form. Conversely, *Triatoma infestans* does not show a linear relationship between SC length and RN number, because each SC has only a single RN and the SCs vary in length (SOLARI and AGOPIAN 1987).

TABLE 5

Distribution of RNs between SC in euchromatin and SC in heterochromatin

SC no.	Mean length (μm)	Percent length of SC set	Euchromatin: Mean length (μm)	Euchromatin: Mean no. of RNs	Euchromatin: (A) Mean no. of RNs/μm	Heterochromatin: Mean length (μm)	Heterochromatin: Mean no. of RNs	Heterochromatin: (B) Mean no. of RNs/μm	Ratio of (A) to (B)
1	30.0	13.07	22.5	2.44	0.112	7.5	0.04	0.005	22.4
2[a]	21.3	9.28	17.1	2.05	0.122	4.2	0.03	0.007	17.4
3	23.1	10.07	16.0	2.07	0.131	7.1	0.03	0.004	32.8
4	20.8	9.06	13.7	1.87	0.138	7.1	0.02	0.003	46.0
5	16.2	7.06	9.5	1.63	0.168	6.7	0.04	0.006	28.0
6	18.5	8.06	12.9	1.69	0.132	5.6	0.04	0.007	18.8
7	18.5	8.06	11.6	1.75	0.155	6.9	0.02	0.003	51.7
8	18.5	8.06	11.9	1.66	0.143	6.6	0.02	0.003	47.7
9	16.2	7.06	10.0	1.58	0.160	6.2	0.004	0.0006	266.7
10	16.2	7.06	10.0	1.64	0.160	6.2	0.02	0.003	53.3
11	16.2	7.06	9.7	1.63	0.165	6.5	0.03	0.005	33.0
12	14.0	6.10	8.2	1.54	0.183	5.8	0.05	0.009	20.3

[a] Because the length of the short arm of chromosome 2 is not known, no total SC length is given for chromosome *2* and the mean length refers to the long arm of chromosome *2* only.

However, in this insect most of the variation in SC length is due to variation in the amount of heterochromatin. (See discussion below about inertness of heterochromatin.)

In addition, in tomato there is a strong linear correlation between the average number of RNs per arm and the average length of SC arms (excluding the heterochromatic short arm of chromosome *2*, $r^2 = 0.96$, $y = 0.091x + 0.044$, $P = 0.0001$; Table 7, Figure 15). LEVAN (1934) also reported a strong correlation between arm lengths and chiasma numbers in *A. macranthum*.

On the other hand, when the absolute combined lengths of the SCs in sets are plotted against the number of RNs in the sets, there appears to be only a weak tendency for longer sets of SCs to have more RNs ($r^2 = 0.18$, $P = 0.0001$). Because the relationship is not strong, there must be other controls over the number of RNs per SC set. Likewise, RASMUSSEN and HOLM (1978) found no correlation between total length of SC sets and number of RNs in humans.

TABLE 6

RNs observed in heterochromatin *vs.* RNs shown in heterochromatin in Figure 12 and in APPENDIX

SC no.	Observed	Histograms
1	16	58
2	12	54
3	13	59
4	10	52
5	18	51
6	16	44
7	7	52
8	10	45
9	2	36
10	7	55
11	12	48
12	19	63

RN numbers are primarily related to the length of SC in euchromatin: Because there are few RNs on SCs in heterochromatin, one might expect an even better correlation between the average number of RNs in euchromatin and the average length of each SC in euchromatin. This relationship is strong ($r^2 = 0.95$, $y = 0.067x + 0.94$, $P < 0.0001$; Table 5, Figure 14), but no stronger than the relationship between the average number of RNs on each SC and the average length of whole SCs (see above, $r^2 = 0.96$). This is probably due in large

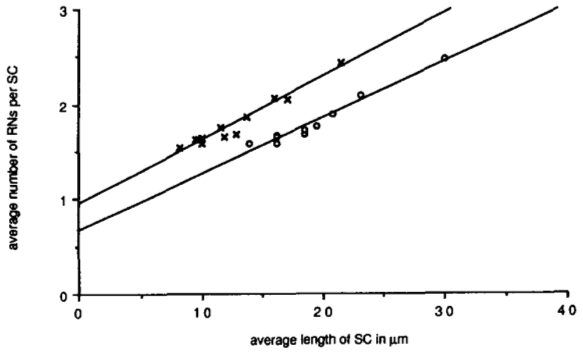


FIGURE 14.—Linear regressions comparing the average number of RNs on each SC with the average length of each SC (○) and the average length of each SC in euchromatin (×) (data shown in Tables 4 and 5). There are only nine points visible for the "○" line because some of its points overlap and SC *2* was not included because we do not know its complete length. When the lengths of SC in heterochromatin are removed to yield the "×" line, the × regression line shifts to the left with nearly the same slope. Note that the × regression line has a *y* intercept close to 1, indicating that if the length of SC in euchromatin were reduced to 0, there would still be one RN on each SC.

TABLE 7

SC arm lengths and RNs per arm

SC no.	Short arm				Long arm			
	Mean length of SC (μm)	Mean no. RNs	Mean length of SC in euthromatin (μm)	Mean no. of RNs on SC in euchromatin	Mean length of SC (μm)	Mean no. RNs	Mean length of SC in euthromatin (μm)	Mean no. of RNs on SC in euchromatin
1	7.5	0.58	3.8	0.56	22.5	1.90	17.8	1.89
2	—	—	—	—	21.5	2.08	17.1	2.05
3	5.3	0.51	3.2	0.48	17.9	1.59	12.8	1.59
4	5.6	0.53	3.3	0.52	15.2	1.36	10.4	1.34
5	7.9	0.82	4.8	0.80	8.3	0.85	4.8	0.83
6	4.3	0.27	2.2	0.27	14.2	1.46	10.7	1.43
7	6.7	0.58	3.9	0.58	11.8	1.19	7.7	1.17
8	5.5	0.44	3.0	0.42	13.0	1.24	8.9	1.23
9	5.8	0.52	3.4	0.52	10.4	1.06	6.6	1.06
10	5.2	0.47	2.8	0.46	11.0	1.19	7.2	1.18
11	7.3	0.79	4.6	0.78	8.9	0.87	5.1	0.85
12	6.9	0.78	4.0	0.76	7.1	0.81	4.2	0.79

part to the fact that for each chromosome, the average length of SC in euchromatin is correlated with the average length of SC in heterochromatin ($r^2 = 0.88$, Table 5). Thus, the presence of a largely inert segment of SC (in heterochromatin) that is proportional to the length of SC in euchromatin has little effect on the correlation between the length of SCs and the number of RNs they carry.

The linear relationship between the average number of RNs on SCs in euchromatin and the average length of SC in euchromatin extends to tomato SC arms as well ($r^2 = 0.93$, $y = 0.11x + 0.02$, $P < 0.0001$; Table 7, Figure 15).

The y intercept of the regression line for average SC length in euchromatin *vs.* average number of RNs on SCs in euchromatin is 0.94 (Figure 14). This indicates that if the amount of SC in euchromatin were reduced to 0, there would still be an average of 0.94 nodules (~1) RN per SC. In comparison, the y intercept for the regression line for average SC length (including heterochromatin) versus average number of RNs per SC is 0.67 RNs (Figure 14). This lower y intercept is due to adding SC length (in heterochromatin) to increase the overall length of SCs with little affect on RN numbers. Thus, the points for the former regression line are simply shifted to the right to give the points for the latter regression line. Again this is an aspect of the relative inertness of SC in heterochromatin.

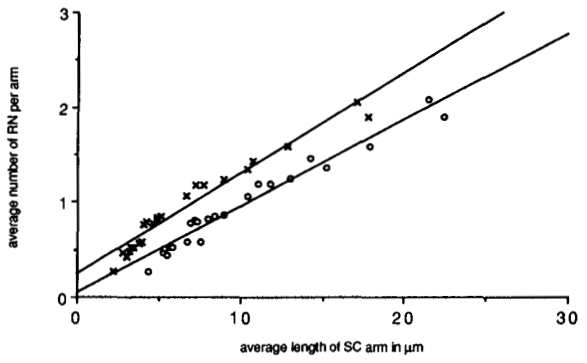


FIGURE 15.—Linear regressions comparing the average number of RNs per SC arm with the average length of each SC arm (○) and comparing the average number of RNs in euchromatin for each SC arm with the average length of euchromatin in each SC arm (×) (data shown in Table 7). Note that when the lengths of SC in heterochromatin are removed to yield the × line, the × regression line shifts to the left with nearly the same slope.

The observation that in tomato there is at least one RN per SC regardless of SC length is consistent with the report by RAHN and SOLARI (1986) that in the chicken, the SCs of every pair of microchromosomes have a RN (sometimes two RNs) in spite of their short length and the report by SOLARI and AGOPIAN (1987) that in the insect *T. infestans* every SC has a RN regardless of SC length. Similarly, other organisms that show great disparity in chromosome size within their complements such as *S. parallelus, L. migratoria,* and *Y. flaccida* likewise have at least one chiasma per chromosome regardless of chromosome length (MATHER 1938). Such observations support a model where each SC has at least one RN with subsequent RNs occurring in proportion to the length of SC in euchromatin. A corollary of this relationship is that short SCs will average more RNs per unit length than longer SCs (Table 5; also for similar conclusion related to *S. commune* and *Saccharomyces cerevisiae,* see CARMI *et al.* 1978 and KABACK *et al.* 1992, respectively).

If DNA is packaged the same way in both short and long SCs, short chromosomes will have more crossovers (and map units) per kilo base of DNA than long chromosomes. This observation could be important for molecular studies using chromosome walking to find genes because a map unit on short chromosomes will represent fewer kilobases of DNA than a map unit on long chromosomes.

TABLE 8

Observed and expected distribution of RNs on SC arms based on the length of SC in euchromatin

SC no.	Mean euchromatic SC length in long arm/ short arm (μm)	1 RN/SC observed		2 RNs/SC observed		3 RNs/SC observed		4 RNs/SC observed	
		On long arm	On short arm	On one arm	On both arms	On one arm	On both arms	On one arm	On both arms
1	17.7/3.7	70 (58)	0 (12)	126 (141)	70 (55)	35 (95)	130 (70)	3 (12)	23 (14)
2[a]	17.1/—	161	0	245	0	43	0	4	0
3	12.8/3.2	114 (87)	0 (27)	121 (167)	125 (79)	12 (35)	55 (32)	1 (5)	10 (6)
4	10.4/3.3	137 (104)	0 (33)	93 (157)	154 (90)	6 (18)	32 (20)	0 (3)	8 (5)
5	4.8/4.8	92 (112)	132 (112)	40 (86)	133 (87)	0 (4)	19 (15)	0 (0)	3 (3)
6	10.7/2.2	202 (168)	0 (34)	126 (149)	81 (58)	7 (18)	25 (14)	0 (1)	3 (2)
7	7.7/3.9	159 (119)	22 (62)	55 (194)	163 (24)	4 (14)	38 (28)	0 (1)	4 (3)
8	8.9/3.0	195 (155)	12 (52)	82 (131)	128 (79)	5 (17)	33 (21)	0 (0)	1 (1)
9	6.9/3.4	156 (132)	44 (68)	46 (111)	157 (46)	0 (6)	18 (12)	0 (0)	1 (1)
10	7.2/2.8	166 (139)	27 (54)	64 (121)	138 (81)	1 (11)	26 (16)	0 (0)	0 (0)
11	5.1/4.6	93 (103)	101 (91)	37 (104)	169 (102)	0 (5)	20 (15)	0 (0)	2 (2)
12	4.2/4.0	82 (97)	108 (93)	46 (97)	148 (97)	1 (9)	33 (25)	0 (0)	0 (0)

Parenthetical values are the expected number of SCs. To determine the expected number of SCs with only one RN that occurs in the long arm, the mean lengths of SC in euchromatin in the long and short arms were added to give a total length of SC in euchromatin. The mean length of euchromatic SC in the long arm was divided by the total to give the fraction of euchromatic SC in the long arm. This fraction was subtracted from 1 to give the fraction of euchromatic SC that occurs in the short arm. These fractions were multiplied by the observed number of SCs with only 1 RN to give the expected number of SCs in which the RN occurs in the long arm and the expected number of SCs in which the RN occurs in the short arm. This calculation was not meaningful for SC *2* that only has euchromatin in its long arm. The expected numbers of SCs with 2, 3, or 4 RNs all in the long arm was determined for each SC by multiplying the number of SCs observed with 2, 3, or 4 RNs by the fraction of euchromatic SC in the long arm that had been raised to the second, third, and fourth power, respectively. This operation was repeated for the short arm to give the expected number of SCs with 2, 3, or 4 RNs in the short arm only. The expected numbers of SCs with 2, 3, or 4 RNs in the long arm were added to the respective numbers of SCs with 2, 3, or 4 RNs in the short arm to give the expected number of SCs with 2, 3, or 4 RNs in one arm only.

[a] Because the short arm of chromosome *2* is heterochromatic and often desynapsed and fragmented, its length is not accurately known. RNs were never observed in the short arm of SC *2*.

It is interesting that the rule that each pachytene bivalent will have at least one RN does not apply to chromosome arms, because if it did, each arm would have at least one RN. Thus, whatever the nature of the control on RN numbers, it applies to whole chromosomes and must extend across centromeres.

Single RNs are found in the long arms of SCs more often than would be expected on the basis of arm length: In cases where subacrocentric SCs (*1, 2, 3, 4,* and *6*) had only one RN, the single RN invariably was found on the long arm . This is expected in chromosome *2* where RNs were not observed on SCs in the heterochromatic short arm. However, for the remainder of the subacrocentrics, one might expect single RNs to be distributed on the basis of the relative length of SC in euchromatin in the short and long arms. For instance, because the euchromatic part of SC *1*'s short arm is 17% of the total length of SC in euchromatin, one might predict that single RNs would lie in the short arm a similar percentage of the time. Instead, single RNs were never found in the short arm of this or any of the other subacrocentric SCs (Table 8). Similarly, for the submetacentric SCs (*7, 8, 9* and *10*), fewer RNs occurred in short arms than would be predicted by their relative euchromatic lengths (*t*-test from SIMPSON *et al.* 1960; $P = 0.0005$, Table 8). Finally in the case of metacentric SCs (*5, 11,* and *12*), which have little or no difference in the euchromatic length of their short and long arms, here again a single RN occurs more often in one arm than would be predicted by arm length (but not necessarily favoring the slightly longer arm, $P = 0.005$, 0.05, and 0.025, respectively; Table 8).

These observations might be explained if crossing over occurs preferentially near synaptic initiation sites (RASMUSSEN and HOLM 1978; STACK and ANDERSON 1986a; ZICKLER *et al.* 1992) and if synapsis is always initiated in long arms of the subacrocentric bivalents, usually initiated in long arms of submetacentric bivalents, and usually initiated in one of the two arms of metacentric bivalents.

Patterns of multiple RNs on SCs indicate crossover interference: As might be expected, longer SCs have more than one RN more often than shorter SCs (Table 4). If there are two or more RNs on the same SC, RNs occur in both arms more frequently than would be expected if RNs were distributed in proportion to the length of SC in euchromatin (*t*-test from SIMPSON *et al.* 1960; $P > 0.0005$, Table 8). HOLM (1987) noted similar distributions of RNs on SCs from Coprinus and humans. These observations suggest that the occurrence of a RN in one arm tends to drive another RN into the other arm. This can be interpreted as positive crossover interference, *i.e.,* the occurrence of one crossover inhibits nearby crossovers. However, in spite of a strong bias

TABLE 9

Average distance between two RNs on the same arm in euchromatin

	Short arm				Long arm			
SC no.	Mean SC length in euchromatin (μm)[a]	No. of observations	Observed mean distance between RNs (μm)	Expected mean distance between RNs (μm)[b]	Mean SC length in euchromatin (μm)[a]	No. of observations	Observed mean distance between RNs (μm)	Expected mean distance between RNs (μm)[b]
1	3.8	4	1.30 ± 1.22 (0.3–3.0)	1.27	17.8	247	8.75 ± 4.64 (0.5–18.5)	5.93
2	—	0	—	—	17.1	236	7.05 ± 3.50 (0.5–17.1)	5.67
3	3.2	4	1.79 ± 1.34 (0.4–3.2)	1.10	12.8	171	5.35 ± 2.83 (0.6–13.8)	4.23
4	3.3	6	1.63 ± 0.80 (0.6–2.4)	1.13	10.4	119	4.58 ± 2.20 (0.6–9.3)	3.40
5	4.8	26	1.98 ± 0.82 (0.3–3.6)	1.60	4.8	35	2.11 ± 1.06 (0.4–4.2)	1.56
6	2.2	0	—	0.73	10.7	141	4.63 ± 2.22 (0.9–10.2)	3.57
7	3.9	6	1.13 ± 0.72 (0.5–2.2)	1.33	7.7	81	3.16 ± 1.83 (0.4–7.4)	2.63
8	3.0	8	1.31 ± 0.92 (0.4–2.3)	1.03	8.9	104	3.81 ± 1.71 (0.6–7.4)	2.93
9	3.4	4	1.20 ± 0.89 (0.5–2.1)	1.67	6.6	57	2.76 ± 1.46 (0.5–6.1)	2.27
10	2.8	4	1.91 ± 0.15 (1.7–2.0)	0.93	7.2	79	3.30 ± 1.52 (0.5–6.9)	2.40
11	4.6	17	1.81 ± 0.81 (0.5–6.9)	1.53	5.1	35	2.02 ± 1.13 (0.4–4.8)	1.70
12	4.0	26	1.70 ± 1.11 (0.3–4.1)	1.37	4.2	30	1.53 ± 0.78 (0.4–3.2)	1.37

Only SCs arms with two RNs in euchromatin were included in this analysis. Observed distances between RNs are means ± SD, with ranges in parentheses.

[a] Mean arm length in euchromatin was calculated on the basis of the mean length of a complete set of SCs being 229.5 μm [Table 4 and the arm ratios and percent of each arm that is heterochromatic presented in Table 2 in SHERMAN and STACK (1992)].

[b] The expected mean distance between RNs on SCs in euchromatin is $^1/_3$ the length of SC in euchromatin.

in favor of a single RN in the long arm of a bivalent, additional RNs occur in short (and long arms) often enough to assure that the average number of RNs in an arm is closely correlated with the length of SC in euchromatin (Table 5, $r^2 = 0.93$).

The average distance between two RNs present in the same arm varies directly according to the length of euchromatic SC in the arm: Thus, longer arms have longer average distances between RNs ($r^2 = 0.97$, $y = -0.12537 + 0.45015x$, $P < 0.0001$; Table 9, Figure 16). Based on random placement of adjacent RNs on SCs in euchromatin, the expected mean separation is $^1/_3$ of the length of SC in euchromatin. The rationale for using $^1/_3$ is that if two particles (RNs) fall at random on a line (SC), on average the particles will divide the line into three equal parts, which means that the two particles will average being separated by $^1/_3$ of the length of the line (CARPENTER 1988 and see MATERIALS AND METHODS). The observed mean separation was not significantly different from this expected mean for any SC because the observed mean separations were within one standard deviation of the expected. However, the observed means were consistently larger than the expected (except for the short arms of chromosomes *7* and *9*), which suggests crossover (RN) interference (Table 9).

When there are only two RNs in euchromatic long arms of SCs, the RNs show both positive and negative interference: Each pair of RNs was classified as separated by ≤0.1, 0.1–0.2, 0.2–0.3, 0.3–0.4, 0.4–0.5, 0.5–0.6, 0.6–0.7, ≥0.8 of the euchromatic SC length of the long arm (Table 10). The observations were grouped in these separation intervals so that there were enough observations in each category to make statistical comparisons. Only long arms with two RNs were analyzed in this manner because short arms rarely had two RNs. When the numbers of observations in each category were graphed for each SC, the curves all have the same general shape with the mode skewed to the left. The shape of these curves is illustrated by combining the data from the long arms of all 12 SCs (Figure 17).

If RNs are randomly and independently positioned in euchromatic segments of long arms, the expected frequency of RN pairs in the various separation intervals is illustrated as the straight line in Figure 17. A chi-squared comparison of the observed frequency of RN pair separations with the expected frequency of RN pair separations based on independent and random placement of each RN in a pair indicates that the two curves are significantly different for the combined data ($P < 0.01$) and every SC individually ($P < 0.05$). This means that the assumptions of random distribution and/or independent placement of each RN in pairs are violated by the observed frequencies of intervals between pairs of RNs.

Compared with expected separation distances between two RNs in the same arm based on random and independent placement (Figure 17), there are too few observed RN pairs separated by an interval of ≤0.1 of the euchromatic arm, too many observed RN pairs separated by an interval of 0.1–0.5 of the euchromatic arm, and too few observed RN pairs separated by an interval of 0.5–1.0 of the euchromatic arm. This can be interpreted as positive interference in the ≤0.1 and the 0.5–1.0 intervals and/or negative interference in the 0.1–0.5 intervals.

In any case, a remarkable aspect of interference is that the long arm of every SC shows essentially the same pattern of interference over a fourfold difference in long arm lengths (Table 7). This suggests that whatever the nature of interference, it is not exerted over an absolute physical distance that applies to all chromosomes in a set, but rather interference is somehow modulated according to chromosome length. The suggestion that the strength of interference varies from SC to SC is supported by the observation that the mean distance between pairs of RNs is directly related to SC length (Table 9).

Other investigators have found RN pairs to be farther apart than expected based on a random distribution, and this has been interpreted as an effect of crossover interference (Rasmussen and Holm 1978; Glamann 1986; Holm *et al.* 1981).

While the shortest observed distance between two RNs was 0.3 μm (Table 9), it is unclear whether 0.3 μm represents a lower limit for adjacent RNs. In this regard, it may be significant that adjacent early (zygotene) nodules can actually touch each other (Stack and Anderson 1986a; see Rasmussen and Holm 1978, 1984 for similar observations of RNs on human SCs).

RNs are not randomly distributed along SC arms: As discussed earlier, RNs occur predominantly on SCs in euchromatin (Figure 12). Aside from segments of SCs near telomeres, there are only 10 0.1-μm segments of SC in euchromatin where RNs were not observed (out of a total of 1526 such segments). Because of this, we think the absence of RNs in these segments is probably due to an inadequate sample size, *i.e.*, RNs probably can occur anywhere along SCs in most euchromatin.

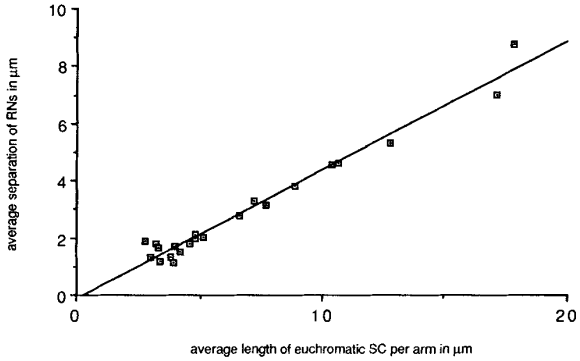


Figure 16.—Linear regression comparing the average distance between two RNs on SCs in euchromatin in one arm with the average length of SC in euchromatin in the arm (data shown in Table 9).

However, this is not to say that RNs are evenly distributed in euchromatin. For example, there are no RNs near telomeres, and there are fewer RNs per unit length of SC near euchromatin/heterochromatin borders (Figure 12). Also there appear to be variations in the distribution of RNs in the remaining segments of SC in euchromatin (Figure 12). It is not clear whether some, all, or any of this latter variation in RN distribution is real (see below). Apparent variations in RN distribution might disappear with larger sample sizes, or variations could be related to crossover hot spots, phenomena that have been reported at the molecular level in some fungi (*e.g.*, Cao *et al.* 1990; Stapleton and Petes 1991) and mammals (for reviews, see Steinmetz *et al.* 1987; Lindahl 1991).

For heterochromatin, the pattern is that RNs are increasingly common further from kinetochores and approaching euchromatin/heterochromatin borders (Figure 12).

In reference to other species, RNs can be highly localized along SCs as in the cases of *S. macrospora* (Zickler 1977), *Physarum polycephalum* (Lie and Laane 1982), *A. fistulosum* (Albini and Jones 1988), *T. infestans* (Solari and Agopian 1987), and *A. porrum* (Stack 1993). On the other hand, it is probably more common for RNs not to be highly localized while still being nonrandomly distributed, as is the case for tomato, *S. commune*, (Carmi *et al.* 1978), *N. crassa* (Gillies 1979), *B. mori* (Holm and Rasmussen 1980), *C. cinereus* (Holm *et al.* 1981), *S. humana* (Zickler and Sage 1981), humans (Solari 1982; Bojko 1985), a Lolium hybrid (Jenkins 1985), *G. domesticus* (Rahn and Solari 1986), mouse (Glamann 1986), and *Mesotoma ehrenbergii ehrenbergii* (Croft and Jones 1989).

Excluding telomeres and euchromatin/heterochromatin borders, RNs may occur randomly on SCs in euchromatin: As one test for random distribution of RNs on SCs in euchromatin, we first determined the cumulative frequency of RNs on each 0.1-μm segment in the long arm of each SC (see Figure 12 and Appendix).

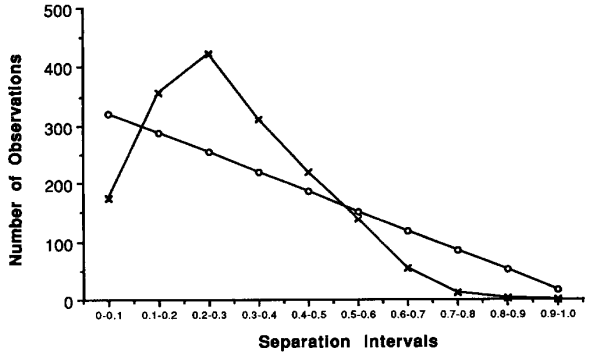


FIGURE 17.—Observed (×) and expected (○) distributions of separation intervals between pairs of RNs in the long arms of tomato's 12 SCs. Intervals are expressed as fractions of the length of SC in euchromatin in long arms. Long arms with only two RNs were used in this comparison. The distances (intervals) between 1687 RN pairs were measured. The expected distribution is based on the assumptions of random and independent location of both RNs in a pair (see MATERIALS AND METHODS). The expected fraction for each separation interval was multiplied by the total number of observations for all 12 SCs (1687) to yield the expected number of RN pairs in each interval. When data for RN pairs from the long arm of each SC were plotted separately and compared with the expected frequencies, all observed curves had the same general shape as the illustrated curve for the combined data.

Then for each long arm, we tallied the number of 0.1-μm segments carrying 0, 1, 2, 3, *etc.*, RNs. Because of the shortage of RNs near euchromatin/heterochromatin borders and telomeres, these segments of SCs were not included in this analysis. For example, the analysis of SC *1* began in the long arm 4.1 μm from the kinetochore and ended 0.4 μm from the end. In this case, we examined 457 long arms and found that among the 159 segments of SC in euchromatin, there were no segments with no RNs, four segments with one RN, 13 segments with two RNs, 24 segments with three RNs, *etc.* (see APPENDIX). For this SC *1* data set, the total number of RNs observed in the 159 segments was 891 to yield an average of (891 ÷ 159) 5.60 RNs per segment with a standard deviation of 2.65. If RNs were randomly distributed on SCs in euchromatin, then 0.1-μm segments with the average number of nodules would be the most frequent class while the remainder of the segments would have more or less RNs in frequencies that form a normal bell-shaped curve (ZAR 1984). We used the means and standard deviations of RNs per segment for each SC to generate normal curves and expected numbers of segments with different numbers of RNs for each SC data set. When chi-squared tests were used to compare the observed frequencies of 0.1-μm segments with different numbers of RNs with the expected frequencies of 0.1-μm segments with different numbers of RNs, only SC *4* ($P = 0.04$) and SC *11* ($P = 0.0002$) showed significant differences from the normal distributions. Even for SC *4* and *11*, we doubt the data sets are really different from normal because the distributions of segments for the other 10 SCs are indistinguishable from normal distributions, the distribution for SC *4* is only marginally significantly different from normal, and the distribution for SC *11* is similar to the distributions for the other SCs except for the absence of segments with five RNs and the relative abundance of segments with four RNs.

In conclusion, these observations seem compatible with random distribution of RNs along most of the length of SC in euchromatin. However, this analysis does not rule out nonrandomness that is due to an abundance of nodules in some contiguous SC segments with compensating deficits in other contiguous SC segments (see above).

RN maps are physical maps of recombination: On classical linkage maps, two linked genes that recombine 1% of the time during meiosis are separated by 1 map unit, and 50 map units correspond to a segment of a chromosome where an average of one crossover occurs per meiosis. If a RN corresponds to a crossover event, then a segment of a SC that averages one RN within its

TABLE 10

Observed pairs of RNs separated by intervals expressed as fractions of the length euchromatic length of the SC arms in which they occur

SC no.	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	≥0.8
1	42	65	86	59	51	42	19	2	2
2	24	67	81	63	47	29	18	4	1
3	26	53	51	46	29	16	5	2	0
4	14	28	35	30	21	10	2	0	0
5	6	5	12	7	4	1	0	0	0
6	12	32	45	30	21	13	6	3	0
7	13	24	25	11	9	6	3	0	0
8	9	28	32	24	12	10	0	0	0
9	7	19	16	11	5	5	0	0	0
10	7	17	17	19	15	5	1	0	0
11	7	13	12	2	5	1	0	0	0
12	7	6	10	9	1	0	0	0	0
Total	174	357	422	311	220	138	54	11	3

TABLE 11

Comparison of the lengths of tomato's RN map, classical gene linkage map, and molecular linkage map

SC no.	Mean SC length (μm)	Mean no. RNs/SC	cMs in RN map[a]	cMs in classical gene map[b]	cMs in molecular map[c]
1	30.0	2.48	124.0	161	131.5
2	21.3	2.08	104.0	74	124.2
3	23.1	2.10	105.0	111	126.1
4	20.8	1.89	94.5	89	124.6
5	16.2	1.67	83.5	55	97.4
6	18.5	1.73	86.5	113	101.9
7	18.5	1.77	88.5	71	91.1
8	18.5	1.68	84.0	67	96.9
9	16.2	1.58	79.0	62	111.0
10	16.2	1.66	83.0	132	90.1
11	16.2	1.66	83.0	97	88.0
12	14.0	1.59	79.5	31	93.1
Total	229.5	21.89	1094.5	1063	1275.9

[a] The number of centimorgans (cMs) or map units per SC was determined by multiplying the average number of RNs/SC by 50 cMs/RN.
[b] TANKSLEY and MUTSCHLER (1990).
[c] TANKSLEY *et al.* (1993).

boundaries would also be 50 map units long. A complete set of tomato SCs averages 21.89 RNs, which is (21.89 RNs × 50 map units/RN) 1094.5 map units. Thus, map units can be assigned to each SC and each SC arm. Because both SC length and SC arm length are closely related to RN numbers, SC length and SC arm length are also closely related to map length (Table 11).

The frequency of RNs in each 0.1-μm segment of SC was also converted to map units (see MATERIALS AND METHODS and APPENDIX). To illustrate the recombination map for each SC, we placed a horizontal line (tick) after every map unit along each SC arm starting at the centromere (Figure 18). The observed variation in the distance between lines is another expression of variation in the rate of crossing over along SCs in both euchromatin and heterochromatin. Similarly, TANKSLEY *et al.* (1992) noted gaps in the molecular map that suggest variations in the frequency of crossing over along tomato chromosomes.

RN maps differ from linkage maps: Table 11 shows the average length of each SC, the average number of RNs on each SC, the calculated number of map units for each SC, the number of map units for corresponding classical gene linkage groups (TANKSLEY *et al.* 1992), and the number of map units for corresponding molecular linkage groups [based on RFLPs, RAPDs, and isozyme markers (TANKSLEY *et al.* 1992)]. The classical gene map is shortest at 1063 map units, the RN map is intermediate at 1094.5 map units, and the molecular map is longest at 1275.9 map units. When a pairwise comparison is made between map lengths of individual chromosomes in the three maps, the worst correlation is between the molecular map and the classical map (r^2 = 0.211); the RN map *vs.* the classical map is intermediate (r^2 = 0.455); and the RN map *vs.* the molecular map is best (r^2 = 0.668).

The molecular map must be near saturation with 1030 molecular markers and an average of only 1.3 map units between markers (TANKSLEY *et al.* 1992). In contrast, the smaller size of the classical map is probably due to a lack of terminal markers and unsaturation. The fact that the molecular map is larger than the RN map might be explained if some RNs were naturally or artifactually lost during preparation. However, we think RNs are not being lost for three reasons: there is close agreement between the observed numbers of rod and ring bivalents and the predicted numbers of rod and ring bivalents based on the pattern of RNs on SCs (Table 3), no SCs were observed without RNs, and when the length of each SC is compared with its map length, RN map lengths are much more closely related to SC lengths (SC *2* excluded, r^2 = 0.98, $P < 0.0001$) than SC lengths are related to either the classical map lengths (r^2 = 0.52, P = 0.0124) or the molecular map lengths (r^2 = 0.66, P = 0.0022; also see KHUSH and RICK 1968; TANKSLEY *et al.* 1992).

Is there anything about the way RN maps, classical gene maps, and molecular maps are prepared that could explain their differences in length? It may be important that classical and molecular maps are based on recombination in both primary microsporocytes and primary megasporocytes, while the tomato RN map is based on observations of primary microsporocytes only. In this regard, DE VICENTE and TANKSLEY (1991) used RFLP markers to compare rates of recombination in primary microsporocytes and primary megasporocytes from a *L. esculentum* (tomato) × *L. pennellii* hybrid and found 18% more recombination in female gametes than in male gametes. This suggests that linkage maps relying on data from equal numbers of both male and female gametes should be about (18% ÷ 2) 9% longer than our RN maps based on data from primary mi-

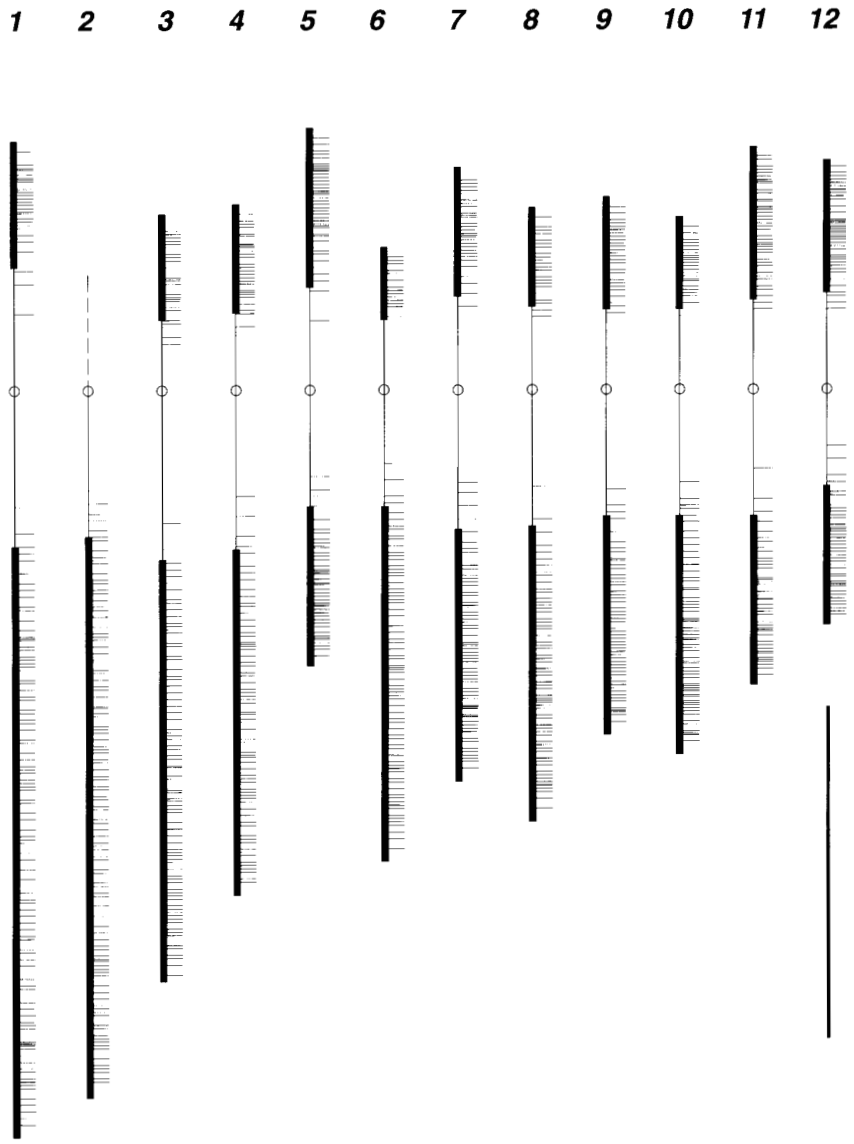


FIGURE 18.—Diagrammatic idiogram of tomato SCs with map units (centiMorgans) superimposed on each SC. SC in euchromatin is represented by a thick line. SC in heterochromatin is represented by a thin line. The kinetochore is represented as ○. The distance between each horizontal thin line equals one map unit measured from the kinetochore. The data used to prepare this diagram are in the APPENDIX. Bar, 10 μm.

crosporocytes only, *i.e.*, 1094.5 × 1.09 = 1193.0 map units, which is closer to the length of the molecular map at 1275.9 map units. Other factors include possible varietal effects on crossover rates (*e.g.*, GAVRILENKO 1984), problems in interpreting map distances involving two or more crossovers between markers, different investigators and techniques, differences in crossover rates between specific markers in primary microsporocytes and primary megasporocytes (DE VICENTE and TANKSLEY 1991; THOMAS and ROTHSTEIN 1991), misclassified phenotypes in RFLP maps that inflate the map (NILSSON et al. 1993), and unknown rates of gene conversion events that might contribute to linkage maps but not RN maps (CARPENTER 1988; STACK *et al.* 1993). Also molecular maps are based on crossover rates in *L. esculentum* × *L. pennellii* hybrids that do not have exactly the same crossover rate and pattern as diploid tomato (RICK 1969). All of these factors, as well as others of which we may not be aware, make linkage maps different from RN maps.

RN maps are a powerful new tool for cytogenetics: RN maps show the physical distribution of crossover events at higher resolution than chiasma maps and without reference to or need for genetic maps. Determining the frequency and position of RNs permits estimates of crossover rates in whole genomes, whole chromosomes, and segments of chromosomes. As a result, it will be relatively easy to determine the effects of factors such as specific genes, polyploidy, chromosomal structures (*e.g.*, NORs, heterochromatin, kinetochores, and telomeres), chromosomal aberrations (duplication, deletions, inversions, and translocations—HERICKHOFF *et al.* 1993; MAGUIRE and RIESS 1994), mutagenic chemicals, irradiation, and environmental factors on crossing over.

Compiling RN maps will be valuable in conducting mutational analyses of genetic regulation of synapsis and crossing over. Coupled with *in situ* hybridization, RN maps will permit estimates of crossover rates between defined molecular markers, which can be compared with cross over rates from linkage maps. This approach is a means of relating RN, genetic, and molecular maps of recombination.

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APPENDIX

Numbers of RNs observed in each 0.1-μm segment of tomato's 12 SCs starting at the end of the shortest arm and progressing to the end of the long arm are shown in Table A1. The length of each SC is the average length shown in Table 11. The series of 0.1-μm segments is indicated in the column to the left (1–300). The position of each kinetochore is indicated by a bold "K" (**K**). Because no RNs were observed in kinetochores, the value of K in all cases is 0. Bold numbers (*e.g.*, **3**) mark the average position of euchromatin/heterochromatin borders, asterisks (*) mark one standard deviation from the indicated euchromatin/heterochromatin border, and pluses (+) mark borders of map units measured from kinetochores. Two pluses mean that two map units fall within that 0.1-μm segment. See MATERIALS AND METHODS for the method of calculating the borders of map units shown as thin horizontal lines on diagrammatic SCs in Figure 18. The number of SCs of each type that were analyzed are shown in parentheses at the beginning of each column.

TABLE A1

Observed distribution of RNs along the 12 tomato SCs

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
1	0	**K**[a]	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	1+
3	3+	0	0	1+	3+	2+	0	2+	2+	5+	3+	2
4	3	0	2	3	6	6+	5+	2	4	5	4+	4+
5	1	0	4+	2+	4+	10+	6+	2	8+	3+	7	8+
6	1	0	11+	8+	5	5	9+	10+	1	4	4+	12+
7	10+	0	7+	9+	7+	4+	10+	4+	7+	4+	8+	7+
8	12++	0	10+	5	9+	9+	7+	8+	5+	9+	12+	9+
9	6	0	9+	11++	4+	3	5	4	12+	12+	9+	4+
10	10+	0	7+	5	5	4+	7+	8+	7+	9+	11++	10+
11	18++	0	5	3	15++	6	5+	10+	12+	10+	8+	7+
12	3+	0	6+	16++	12++	7+	11+	8+	6+	17++	4	8+
13	7	0	7+	4+	9+	5+	5	4	9+	6+	9+	8
14	16++	1	6+	9+	7+	7+	13++	5+	3+	10+	4+	5+
15	5+	0	5	7+	9+	14+	8+	9+	10+	5+	7	9+
16	11+	1	3+	8+	11+	7+	5	10+	9+	14+	10++	5+
17	12+	0	3	6	8+	9+	4+	5+	12+	8+	7	4
18	8+	1	2	8+	10+	6+	14+	10+	10+	6+	13++	14++
19	9+	0	14++	10+	16++	5*	8+	8+	5+	14++	6+	7+
20	11+	0	11+	7+	11+	2	15++	4	7+	5	10+	17++
21	12++	1	6+	9+	9+	2+	6+	6+	5	7+	9+	18++
22	5	0	1	7+	6+	**2**	10+	15+	6+	8+	5+	11+
23	8+	0	6	9+	7+	1	8+	7+	5+	8*+	9+	12++
24	9+	0	8+	8+	7+	3	9+	7+	4	6+	9+	11+
25	8+	1	17++	2	11+	1*	10+	4	9+	3	7+	7+
26	8+	3	4+	14++	9+	0	8+	3*+	10+	4+	4	8+
27	5	0	6	6+	9+	0	8+	5	6+	1	7+	7+
28	4+	0	8*+	9*+	10+	0	9+	8+	5+	**1**	10+	7
29	9+	1	2+	7	9++	0	4	6+	6	5	15++	10++
30	8+	3+	3	2+	12+	0	6+	**4**	6*+	2+	10+	11+
31	4*	3	4	5	4	0	4	5+	3	3	10+	7+
32	2	0	**5**+	5+	9++	0	2	5	8+	1	7+	6+
33	4+	3*	5+	**6**+	2	0	2+	3+	7+	1*	2	13+
34	1	4+	9+	4	7+	1	7*	1*	**1**	1	5+	4*+

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
35	2	0	6	1	6	0	2+	3	4+	0	11+	3
36	2	2	2	2	9+	0	3	2	2	0	8+	7+
37	1	5+	2*+	2+	6+	0	4	0	0	0	2	6
38	**1**	5	5	3*	5+	0	2+	0	0	0	5+	3+
39	1+	6+	4+	1	3	0	**5**	0	2*	0	6+	4
40	6	4	0	1	5+	0	2	0	1	0	11+	**7**+
41	0	1	2	1	6	0	1	0	0	0	4*	5+
42	2	6+	2	1	8*+	0	1+	0	1	1	6+	6
43	1+	3	3	0	1	**K**	3	1	0	0	7+	5+
44	0	**7**+	0	1	4+	0	1*	0	0	0	4	1
45	2*	5+	0	0	3	0	4	0	0	0	5+	4+
46	2	2	0	0	3+	0	0	0	0	0	**2**	1*
47	1	1	0	0	1	0	0	0	0	0	5+	0
48	0	7+	0	0	**4**	0	0	0	0	0	5	2
49	1	4	0	0	3+	0	0	0	0	0	2+	0
50	2	8+	0	0	3	0	0	1	0	0	2	1
51	0	7+	0	0	0	0	0	0	0	0	0	0
52	2+	5+	0	0	0	0	0	0	0	**K**	1*	2
53	0	1	**K**	0	1	0	0	0	0	0	1	1
54	2	4	0	0	1*	0	0	0	0	0	2	0
55	0	6*+	0	0	0	0	0	**K**	0	0	0	0
56	2	5	0	**K**	2	0	0	0	0	0	1	0
57	1	7+	0	0	0	0	0	0	0	0	0	0
58	1	4+	0	0	1+	2	0	0	**K**	0	0	0
59	1	7	0	0	3	0	0	0	0	0	0	0
60	0	8+	0	0	1	2	0	0	0	0	0	0
61	0	7+	0	0	0	0	0	0	0	0	0	0
62	0	4	0	0	0	2	0	0	0	0	0	0
63	0	6+	0	0	0	1	0	0	0	0	0	0
64	1	3	1	0	0	0	0	0	0	0	0	0
65	0	4+	0	0	0	2+	0	0	0	0	0	0
66	0	9+	1	0	1	2	0	0	0	0	0	0
67	0	3	0	0	2	2	**K**	0	0	0	0	0
68	0	4+	0	1	0	1	0	0	0	0	0	0
69	0	0	0	0	1	0*	0	2	0	0	0	**K**
70	0	6	0	0	0	4+	0	0	0	0	0	0
71	0	5+	0	0	0	3	0	0	0	0	0	0
72	0	3	0	0	0	1	0	0	0	0	0	0
73	0	12+	0	0	0	2	0	0	0	1	**K**	0
74	0	6+	0	1	0	2	0	0	0	1	0	0
75	**K**	4+	0	2	0	4+	0	1	0	2	0	0
76	0	4	0	0	0	3	0	0	0	1	0	0
77	0	5+	0	0	0	5+	0	1	1	0	0	1
78	0	5	1	0	0	**5**	0	0	0	1	0	0
79	0	8+	0	1	**K**	2	0	0	0	0*	0	1
80	0	3	0	0	0	3+	0	0	1	4+	1	0

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
81	0	2+	0	1	0	5	2	2	0	0	0	1
82	0	4	0	0	0	5+	0	0	0	1	0	1
83	0	4	0	1	0	4	2	2	1	1	0	2
84	0	7+	0	0	0	9+	0	2+	1	0	0	1
85	0	7+	0	0	0	2+	0	0*	2	7+	0	0
86	0	4	0	1	0	7	1	0	0	4	0	3+
87	0	2+	0	0	0	5*+	0	0	2*	7+	0	0
88	0	2	0	2+	0	6+	0	1	1+	7+	0	3
89	0	10+	3	0	0	1	0	2	3	4	0	2
90	0	6+	0	2*	0	7+	0	3	1	**4**+	0	2+
91	0	3	0*	2	0	4	0	0	1	4	1	0
92	0	5+	2	0	1	11+	0	1	2	3+	0	1*
93	1	1	1+	3	0	6+	2	0	4+	2	1	0
94	0	6	0	3+	1	8+	1	2+	2	3	0	5
95	0	15++	2	0	1	4	1+	5	4+	4+	2	5+
96	0	4	3	0	1	7+	4	**1**	**6**	5	2	4
97	0	5+	1	0	0	11+	2*	1	2+	6+	3+	3+
98	1	6+	1	1	0	8+	3+	4+	2	1	0	**1**
99	1	2	0	4	1	3+	4	1	2	6+	2	3
100	1	6	1	4+	1	4	1	2	4	2	0	6+
101	1	8+	1+	2	0	10+	2	2	3+	6*+	0	9+
102	0	8+	1	2	0	7+	2+	5+	3	5	1	0
103	1	5+	2	3+	0	7+	0	2	1	2+	1*	2
104	0	5	**3**	**4**	2	11+	2	6+	5+	3	1	5*+
105	0	9+	4+	3+	1+	8+	4	3	2*	6+	1	9+
106	1	1	3	1	1	9+	3+	4+	7+	5	3+	11+
107	1	6+	5+	5	3*	9+	4	5*	7+	5+	2	6+
108	0	9+	6	2	3	4	**2**	5+	2	2	1	5+
109	1	8+	4+	5+	3+	3+	2	3	13++	5+	3	7
110	1*+	4	2	4	1	5	3+	9+	5	11+	4+	7+
111	3	6+	6+	4+	6+	3+	2	8+	6+	4	**3**	12++
112	1	5+	4	1	1	4	3	5	9+	9++	0	12+
113	0	3	4+	4+	5	1	4+	3+	4	5	2+	5+
114	1	5+	4	4	**2**+	6+	4	6	8+	1	4	8+
115	1	8	4	4	0	5	7+	10+	4+	7+	2	11+
116	1	6+	5+	3+	2	4+	5+	7+	9+	8+	4+	6+
117	1	4+	4*+	2	4	6	2	1	8+	11+	4	9+
118	3+	7	8	5*+	2+	4+	1	7+	5	7+	7+	9+
119	2	8+	6+	6	2	7+	4*	8+	9+	3+	8+	9+
120	3	4+	3	6+	4	5	6+	10+	6+	8	4*	7+
121	1	5	7+	5+	7*+	6+	6+	5+	6+	11++	7+	11+
122	**7**+	9+	2	7	4+	4	6	9+	6+	7+	4+	10+
123	1	5+	6+	1+	4	6+	7+	4	7	5	4	4+
124	3+	7	3+	6	7+	5+	7+	7+	7+	7+	7+	6
125	4	4+	11+	5+	4	5	8+	11+	7+	10+	8+	10++
126	6+	6+	7+	4	12++	13++	7+	7+	8+	10+	6+	6

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
127	1	3	2	2+	6	16+	7	9+	8+	11++	7+	17++
128	1	5	3	4	8+	5+	3+	6	5+	5	4	13++
129	3	5+	5+	8+	6+	4	8+	3+	7	9+	4	4
130	3+	5+	9+	6+	4+	2+	3	6	9+	8+	11++	7+
131	2	3	4	5	7	3	6+	4+	7+	3+	15++	7+
132	6	8+	2	13++	8+	8+	8+	7+	7+	3	8+	9+
133	3+	6	6+	12+	13++	7+	5	10+	9+	10+	7	10+
134	3	6+	5+	5+	14++	8+	6+	5	5+	11++	8+	8+
135	6*+	0	3	2	6	9+	7+	9+	10+	5	10+	4+
136	4	7+	4	6+	8+	8+	9+	4+	12+	9+	13++	9+
137	6+	8+	4+	6	12++	7	9+	8+	10+	10+	4	4+
138	3	7+	3	4+	4	5+	12+	3	7+	6+	4+	0
139	2	4	6+	7+	8+	5	6+	8+	10+	5+	11+	0
140	8+	5+	6+	5	4+	3+	1	7+	4+	6	8+	0
141	5+	9+	6	7+	11+	5	11+	9+	9+	16++	4+	
142	3	4	4+	6+	10+	4+	4	7	7+	12++	6	
143	2	4	4	1	13++	7+	11++	6+	9+	7+	8+	
144	3+	4+	5+	7+	8+	4	9+	8+	10+	17++	9+	
145	3	6+	4	7	9+	5+	10+	7+	6+	8	12++	
146	3	3	6+	8+	8+	6	11+	8	11+	9++	7+	
147	2+	3	3	7+	11+	7+	10+	8+	8+	13+	8+	
148	8	8+	7+	7+	5+	6+	5+	11++	8+	8+	4	
149	13++	4+	1	9+	9+	5	9+	13+	8+	4+	10+	
150	11+	2	3+	6+	9+	5+	6	5+	8+	9+	6+	
151	3	8+	6	5	4	6+	9+	9+	9+	5	10+	
152	3+	9+	6+	2	5+	5	9+	3	8+	9+	13++	
153	9+	5	3	9++	15++	9+	7+	9+	5	4+	6	
154	6	1	2	4	9+	3	7+	9+	12++	4	10++	
155	5+	4+	8+	7+	8+	7+	7+	4	7+	13++	13+	
156	10+	1	5+	6+	15+	7+	5	6+	7+	9+	4+	
157	9+	2	6+	8	5+	6+	5+	8+	5	4	12+	
158	11+	7+	8+	1+	3	9+	11+	9+	4+	5+	3	
159	5+	5	5	4	4+	6	7+	8+	0	1	3+	
160	1	0	10+	4	0	6+	8+	5	0	0	1	
161	1	4+	5+	4+	0	5+	5	9+	0	0	0	
162	7+	2	5	5+	0	2	11++	7+	0	0	0	
163	7+	5+	5+	3		7+	8+	7+				
164	8	4	5+	5		6	8	11+				
165	5+	6+	5	7+		7+	10++	4				
166	4	5	12++	16++		6+	6	10+				
167	5+	7+	2	1		11+	6+	5+				
168	9+	10+	4	5+		11+	8+	8+				
169	3	3	9+	5		5+	9+	5				
170	2	8+	5+	4+		5	7+	9+				
171	7+	14++	2	5		8+	3	7+				
172	3+	3	4	4+		6+	9+	8+				

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
173	6	6+	13++	8+		10+	7+	7+				
174	11+	9+	5+	6+		6+	4	10+				
175	5+	13+	7	4		6	9+	7+				
176	3	7+	12++	6+		7+	9+	12+				
177	3+	1	5	10+		2	7+	2				
178	2	3	9+	12+		1+	8+	3+				
179	2	3+	5+	3+		4	6+	6				
180	6+	5	9+	10+		1	6	2				
181	8+	3+	2	1		1+	2+	2+				
182	2	6	3	7+		0	0	0				
183	6	6+	5+	2		0	0	0				
184	2+	4	1	6+		0	0	0				
185	3	5+	6+	6		0	0	0				
186	6+	10+	3	9+								
187	6	6+	5+	3+								
188	6+	7+	2	3								
189	6+	0	9+	2								
190	12+	4	3	9+								
191	4	9+	7+	7+								
192	9+	6+	6+	5+								
193	9+	1	9+	4								
194	5+	12+	9+	10+								
195	6+	10+	6+	6+								
196	3	11+	4	4+								
197	4	7+	5+	7								
198	6+	4+	7	8+								
199	5+	5	9+	10+								
200	4	2	8+	4+								
201	4	5+	7+	6+								
202	4+	3	3	6								
203	5	9+	8+	6+								
204	4+	5+	7+	3+								
205	1	9+	9+	0								
206	1	4	6+	0								
207	9+	7+	5	0								
208	2	3+	6+	0								
209	7+	0	6+									
210	5+	0	7+									
211	9+	0	8+									
212	3	0	11+									
213	3	0	9+									
214	3+		2									
215	4		7+									
216	8+		6+									
217	2		9+									
218	3		2									

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
219	2+		5									
220	3		6+									
221	2		7+									
222	4+		4									
223	3		6+									
224	1		7+									
225	3		6									
226	7+		1+									
227	3		4									
228	4+		3									
229	6+		1+									
230	4		0									
231	7+		0									
232	4											
233	3+											
234	6											
235	4+											
236	3											
237	8+											
238	6											
239	7+											
240	3+											
241	2											
242	3											
243	8+											
244	5+											
245	7											
246	5+											
247	5											
248	3+											
249	3											
250	1											
251	1											
252	5+											
253	6											
254	4+											
255	6+											
256	0											
257	3											
258	5											
259	1+											
260	2											
261	8+											
262	5											
263	5+											
264	3											

TABLE A1

Continued

0.1-μm segment	SC *1* (457)	SC *2* (453)	SC *3* (438)	SC *4* (430)	SC *5* (419)	SC *6* (444)	SC *7* (445)	SC *8* (456)	SC *9* (424)	SC *10* (422)	SC *11* (422)	SC *12* (418)
265	8+											
266	7+											
267	8+											
268	1											
269	2											
270	5											
271	10++											
272	11+											
273	8+											
274	4											
275	7+											
276	4											
277	4+											
278	5											
279	8+											
280	6+											
281	4											
282	7+											
283	10+											
284	5+											
285	10+											
286	3											
287	3											
288	4+											
289	5											
290	6+											
291	2											
292	6+											
293	5											
294	6+											
295	3											
296	1+											
297	2											
298	0											
299	0											
300	0											

[a] Because the length of the short heterochromatic arm of SC *2* is unknown and RNs were never seen in this arm, the 0.1-μm segments for SC *2* start at the kinetochore.