

# High-Resolution Crossover Maps for Each Bivalent of *Zea mays* Using Recombination Nodules

Lorinda K. Anderson,<sup>\*,1</sup> Gregory G. Doyle,<sup>†</sup> Brian Brigham,<sup>\*</sup> Jenna Carter,<sup>\*</sup> Kristina D. Hooker,<sup>\*</sup> Ann Lai,<sup>\*</sup> Mindy Rice<sup>\*</sup> and Stephen M. Stack<sup>\*</sup>

<sup>\*</sup>Department of Biology, Colorado State University, Fort Collins, Colorado 80523 and

<sup>†</sup>Department of Agronomy, University of Missouri, Columbia, Missouri 65211

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## ABSTRACT

Recombination nodules (RNs) are closely correlated with crossing over, and, because they are observed by electron microscopy of synaptonemal complexes (SCs) in extended pachytene chromosomes, RNs provide the highest-resolution cytological marker currently available for defining the frequency and distribution of crossovers along the length of chromosomes. Using the maize inbred line KYS, we prepared an SC karyotype in which each SC was identified by relative length and arm ratio and related to the proper linkage group using inversion heterozygotes. We mapped 4267 RNs on 2080 identified SCs to produce high-resolution maps of RN frequency and distribution on each bivalent. RN frequencies are closely correlated with both chiasma frequencies and SC length. The total length of the RN recombination map is about twofold shorter than that of most maize linkage maps, but there is good correspondence between the relative lengths of the different maps when individual bivalents are considered. Each bivalent has a unique distribution of crossing over, but all bivalents share a high frequency of distal RNs and a severe reduction of RNs at and near kinetochores. The frequency of RNs at knobs is either similar to or higher than the average frequency of RNs along the SCs. These RN maps represent an independent measure of crossing over along maize bivalents.

**M**AIZE (*Zea mays* L.) is one of the first model organisms in which the power of genetics was productively merged with cytology to create the new field of cytogenetics (reviewed by RHOADES 1984). The ability to recognize all 10 maize chromosomes in pachytene squash preparations was key for relating each chromosome to a specific linkage group (MCCLINTOCK 1931, 1933; RHOADES and MCCLINTOCK 1935). Subsequently, maize chromosome cytology has remained a fertile field for investigation (*e.g.*, DAWE *et al.* 1994; FREELING and WALBOT 1994; BASS *et al.* 2000; MUEHLBAUER *et al.* 2000; SADDER and WEBER 2002) that has been accompanied by the development of sophisticated maize linkage maps (*e.g.*, DAVIS *et al.* 1999; SHAROPOVA *et al.* 2002). However, integration of linkage maps with chromosome structure has been difficult in maize (as well as in other organisms) because crossing over is not evenly distributed along the length of chromosomes. For example, crossing over is typically uncommon in heterochromatin and centromeres compared to euchromatin (*e.g.*, MATHER 1939; for reviews see COMINGS 1972; RESNICK 1987), and even in euchromatin, where the bulk of crossing over occurs, the frequency of crossing over can vary considerably from one part of a chromosome to another (*e.g.*, SOUTH-

ERN 1967; DE LA TORRE *et al.* 1986; JONES 1987; SHERMAN and STACK 1995).

One way to relate linkage maps to the structure of chromosomes is to observe the location and frequency of crossing over along chromosomes by means other than linkage analysis. To a degree this has been accomplished in squash preparations by observing the number and positions of chiasmata on late diplotene-diakinesis bivalents (JONES 1987). Unfortunately, for most species, including maize, this is a relatively inaccurate, low-resolution method because (1) twists can be mistaken for chiasmata, (2) nearby chiasmata may not be resolved, (3) chromosomes are comparatively short when chiasmata are visible, and (4) it is difficult to relate most bivalents to specific pairs of chromosomes (STACK *et al.* 1989; SHERMAN and STACK 1995; STEVENSON *et al.* 1998). Another method for cytologically assessing crossing over has been developed recently using fluorescent antibodies to label MLH1p, a mismatch repair protein that is present at crossover sites during pachytene (BAKER *et al.* 1996; HUNTER and BORTS 1997; ANDERSON *et al.* 1999; MOENS *et al.* 2002). Because MLH1 signals appear as discrete fluorescent foci on pachytene bivalents that are 5–10 times longer than bivalents at diakinesis, MLH1 foci can be mapped at higher resolution than chiasmata can. However, the application of this technique to mapping crossovers has been limited to birds and mammals so far (*e.g.*, PIGOZZI 2001; FROENICKE *et al.* 2002), per-

<sup>1</sup>Corresponding author: Department of Biology, Colorado State University, Fort Collins, CO 80523.  
E-mail: lorinda.anderson@colostate.edu

haps because the antibodies were raised to mammalian MLH1 proteins, and these antibodies do not bind to their plant counterparts (our observations). While representing a significant improvement over chiasmata to map crossover events, analysis of MLH1 fluorescent foci is still limited by the resolution of the light microscope.

The highest-resolution method available to map the frequency and location of crossover events cytologically remains analysis of late recombination nodules (RNs; sometimes abbreviated as LNs) on synaptonemal complexes (SCs; *e.g.*, CARPENTER 1975; reviewed by ZICKLER and KLECKNER 1999; ANDERSON and STACK 2002). RNs are proteinaceous ellipsoids,  $\sim 100$  nm in their longest dimension, which lie on SCs (that is, pachytene bivalents). RNs are closely correlated with crossovers and lie at sites where chiasmata will form later (CARPENTER 1975, 1979; VON WETTSTEIN *et al.* 1984; MARCON and MOENS 2003). The proposed role of RNs as molecular factories for crossing over has been corroborated recently by work showing that the MLH1 protein is a component of late RNs (MOENS *et al.* 2002). The high resolution of RN analysis is due not only to the observation of RNs on relatively long pachytene chromosomes, but also to the small size of RNs compared to chiasmata ( $0.1 \mu\text{m}$  *vs.*  $1 \mu\text{m}$ , respectively) and to the use of electron microscopy to resolve RNs.

The most useful cytological maps of crossing over are those in which every bivalent can be identified unequivocally and related to a specific linkage group. This has not been possible in many organisms, and, in lieu of this, some studies have pooled crossover data from chromosomes of similar size and arm ratios (SOUTHERN 1967; ANDERSON *et al.* 1999). In other studies, it was possible to identify some of the bivalents using either a combination of relative lengths and arm ratios (*e.g.*, LAURIE and JONES 1981; PIGOZZI 2001) or fluorescence *in situ* hybridization of chromosome-specific sequences (LYNN *et al.* 2002; TEASE *et al.* 2002). Thus far, only two studies have mapped crossing over on every bivalent in a set, one using RNs on tomato SCs that were identified by relative lengths, arm ratios, and patterns of heterochromatin (SHERMAN and STACK 1995) and the other using MLH1 foci on mouse autosomal SCs after chromosome-specific painting (FROENICKE *et al.* 2002). Here we report such an analysis in maize. For this, we first prepared an SC karyotype on the basis of relative lengths and arm ratios for the maize inbred line Kansas Yellow Saline (KYS). Each SC was identified and related to a specific maize chromosome and linkage group. Most SC identifications were verified using inversion heterozygotes. We then determined the frequency and distribution of RNs (crossing over) on each of the 10 maize SCs.

#### MATERIALS AND METHODS

**Plants:** Maize inbred KYS and heterozygotes for inversions *1d*, *2i*, *3c*, *4c*, *5d*, *6b*, *7a*, *8c*, *9b*, and *10a* (DOYLE 1994) were

grown in a temperature-controlled greenhouse with supplemental lighting. All inversion heterozygotes were in KYS background.

**Diakinesis chromosome squashes:** Anthers containing diakinesis-stage cells were fixed for 1–24 hr in 1:3 acetic ethanol. After clearing the anthers for 1–5 min in 45% acetic acid, the meiotic cells were squeezed out of the anthers in a fresh drop of 45% acetic acid and squashed lightly under a siliconized cover glass. The cover glass was removed using dry ice, the slide was allowed to air dry, and chromosomes were stained with 2% aceto-orcein under a cover glass with brief heating over an alcohol lamp. After staining, cover glasses were removed by inverting the slide over 95% ethanol. Before the preparations dried, new cover glasses were mounted with Euparal. Complete sets in which all chromosomes were separate and interpretable were photographed using a  $\times 100$  PlanApo objective and a digital camera attached to an Olympus Provis light microscope.

**Pachytene SC spreads:** SC spreads were prepared on plastic-coated slides as described by STACK and ANDERSON (2002). SC spreads were stained with 2% uranyl acetate followed by Reynold's lead citrate (UP) or with 33% silver nitrate (SHERMAN *et al.* 1992). The slides were scanned using phase light microscopy, and good SC spreads on plastic were picked up onto 50- or 75-mesh grids. The grids were examined in an AEI 801 electron microscope, and SC spreads without detectable stretching and with kinetochores were photographed at a magnification of  $\times 1600$  or  $\times 2500$ . In total, 2080 individual SCs from 290 sets were identified and mapped with regard to RNs. Both total RN number and total SC set length (the combined length of all SCs in a cell) could be assessed for 206 of these SC sets. In the remainder of the sets, certain of the SCs could not be identified, usually due to unclear or missing kinetochores. Some SCs had a small amount of asynapsis, particularly near the ends, and may have been in the very earliest stages of diplotene.

**Measurements:** Electron microscope negatives were scanned into a computer using a Hewlett-Packard ScanJet 4c and Adobe Photoshop (version 5.0) software. Montages of SC spreads were assembled using Adobe Photoshop. Proper tracing of each SC and the position of kinetochores and RNs were determined directly from the negatives using a  $\times 8$  magnifying loupe and recorded onto prints of the montages. One lateral element from each SC was measured in micrometers using the computer program MicroMeasure (REEVES 2001). Total SC length varied from set to set, but the relative length of SCs and SC arms, *i.e.*, arm ratios, within each set remained consistent. SCs were identified by relative lengths (percentages of total SC length for the set) and arm ratios (length of the long arm divided by length of the short arm). RNs were recognized using criteria of size, shape, staining intensity, and association with SC as described by STACK and ANDERSON (2002). RN positions were measured and expressed as a percentage of the arm length from the centromere. Using average lengths for each of the 10 SCs and their average arm ratios, each of the SCs was divided into  $0.2\text{-}\mu\text{m}$  segments, and each observed RN was placed into one of these segments on the basis of its original relative position from the centromere. For those SCs in which an arm was not divisible by two, the most proximal interval was made  $0.3 \mu\text{m}$  rather than  $0.2 \mu\text{m}$ . After compiling the RN data, the genetic map length of each SC was calculated by determining the average number of RNs per SC and then multiplying by 50 (one RN = one crossover = 50 cM).

**Statistics:** The program Minitab (version 13) was used for statistical analyses and for preparing histograms. The smoothing (Lowess) lines were based on the histograms (Minitab degree of smoothing = 0.1; steps = 0).

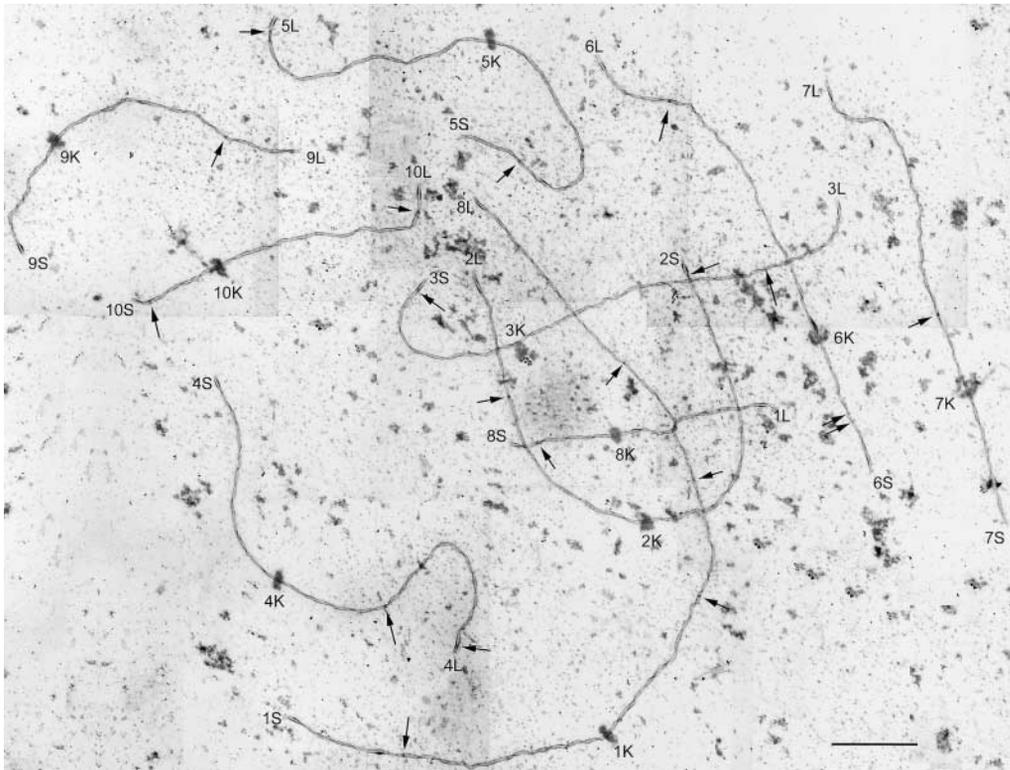


FIGURE 1.—Complete set of silver-stained spread SCs from maize KYS. Each SC has been identified by its relative length and arm ratio and numbered accordingly. Arrows indicate the location of RNs. The two RNs on the short arm of SC 6 are so close together that they would likely result in a structure that would be interpreted as a single chiasma during diakinesis. Lateral element thickenings can be observed on several SCs (e.g., 1S, 2S, 7S, 10L). Such thickenings are more commonly observed with silver staining than with uranyl acetate-lead citrate staining. K, kinetochore; S, short arm; L, long arm. Bar, 5  $\mu$ m.

## RESULTS

**SC karyotype:** SC spreads were prepared by exposing protoplasts to a hypotonic solution containing a small amount of nonionic detergent (STACK and ANDERSON 2002). During this procedure, the cytoplasm as well as the chromatin surrounding each SC decondenses to become almost invisible, while SCs and RNs are relatively unaffected (Figure 1). Dispersion of the chromatin means that such features as chromomeres and knobs are not visible to help identify SCs. In addition, the nucleolus is usually dispersed in these preparations, so it is not possible to detect the association of the nucleolus with the nucleolus organizer (NOR) on the short arm of SC 6 (McCLINTOCK 1934). This means that SC identification must rely on relative length and arm ratio. Arm ratios can be determined only when kinetochores are visible in SC spreads that are probably in mid- to late pachytene.

To prepare the karyotype, SC spreads were selected using the following criteria: (1) each of the 10 SCs could be followed along its entire length, (2) the kinetochore was visible on each SC, and (3) SCs were not visibly stretched. Thirty sets of SCs that met these criteria were measured for lengths and arm ratios. Then SCs in each set were ordered according to their relative lengths. If necessary, the order of an SC was changed so that the arm ratios for each SC were consistent with pachytene maps (Table 1). Out of the ( $10 \times 30 =$ ) 300 SC length positions, 55 (18%) were changed on the basis of the arm ratio data. For most of these changes, the difference

in relative length between 2 (or sometimes 3) SCs was minimal, but the arm ratios were noticeably different. The average relative length and average arm ratio for each ranked SC from the 30 sets are presented in Table 1 along with the same information for five other karyotypes produced from squashes, three-dimensional reconstructions, and SC spreads. Except for absolute total lengths of complete sets and the arm ratio of SC 6 that carries the NOR, the similarity of the karyotypes is striking. Overall, the SCs decrease gradually in average relative length from SC 1 (14.8%) to SC 10 (6.8%), but the length positions of SC 4 and SC 5 have been reversed to reflect the standard pachytene chromosome karyotype. With regard to arm ratios, each ranked SC group is statistically different from the SC group immediately preceding or succeeding it ( $P < 0.002$ , two-sample *t*-test). Thus, each SC in a set can be identified accurately on the basis of its relative length and arm ratio.

To verify that our SC identifications were consistent with the genetic linkage groups, we analyzed spreads of SCs from plants that were heterozygous for one of the following inversions: *1d*, *2i*, *3c*, *4c*, *5d*, *6b*, *7a*, *8c*, *9b*, or *10a* (Figure 2; DOYLE 1994). Unfortunately, obtaining spreads that contained inversion loops along with distinguishable kinetochores on each SC proved to be difficult. For example, no SC spreads from the inversion heterozygotes *In8c* and *In10a* fulfilled these two criteria, and only 27 SC sets (*In1d* = 2; *In2i* = 6; *In3c* = 3; *In4c* = 4; *In5d* = 2; *In6b* = 1; *In7a* = 5; *In9b* = 4) for the other inversion heterozygotes could be used to verify the SC

TABLE 1  
Comparison of selected karyotypes for maize

Chromosome or SC rank	Chromosome squashes		3D fluorescence:		SC spreads	
	McCLINTOCK <i>et al.</i> (1981)	LONGLEY (1939); RHOADES (1950)	DAWE <i>et al.</i> (1992, 1994)	3D sections: GILLIES (1973)	GILLES (1981)	This work
1	14.9 (1.27)	14.8 (1.30)	15.8 (1.30)	14.2 (1.26)	14.9 (1.24)	14.8 (1.27)
2	12.0 (1.20)	12.1 (1.26)	12.3 (1.68)	12.4 (1.20)	11.9 (1.15)	11.9 (1.08)
3	11.2 (2.03)	11.7 (2.01)	10.8 (2.06)	11.1 (2.03)	11.3 (2.06)	11.2 (2.02)
4	10.6 (1.57)	10.6 (1.59)	10.0 (1.39)	10.8 (1.68)	10.5 (1.62)	10.7 (1.56)
5	10.7 (1.16)	10.7 (1.20)	10.1 (1.08)	10.9 (1.21)	11.0 (1.13)	10.8 (1.10)
6	8.9 (3.14)	8.4 (3.22)	9.5 (3.41)	9.2 (3.01)	9.1 (2.75)	8.9 (2.63)
7	8.4 (2.58)	8.3 (2.72)	9.0 (2.38)	8.5 (2.38)	8.8 (2.67)	8.7 (2.76)
8	8.8 (3.29)	8.3 (3.26)	8.2 (2.83)	8.8 (2.92)	8.2 (2.99)	8.5 (3.04)
9	7.7 (1.82)	8.1 (1.85)	7.4 (1.69)	8.0 (1.89)	7.7 (2.09)	7.7 (1.92)
10	6.7 (2.83)	6.9 (2.80)	6.7 (2.31)	6.2 (2.43)	6.5 (2.54)	6.8 (2.43)
Average total length of sets ( $\mu\text{m}$ )	—	556	—	353	421	331
No. of observations	—	28	2	5	14	30

The karyotypes were prepared using a variety of techniques including light microscopy of pachytene chromosome squashes, deconvolution-based, three-dimensional fluorescence light microscopy of intact pachytene nuclei, three-dimensional electron microscopic reconstructions of pachytene nuclei from serial thin sections, or electron microscopy of SC spreads. In all cases, primary microsporocytes from maize inbred KYS were used. For each karyotype, the average relative length of each SC is presented first as a percentage of the total length of the SC set, followed by the average arm ratio in parentheses.

identifications (Figure 2). Because an inversion loop with associated asynapsis on its borders can alter the expected relative length and arm ratio for the inversion loop SC, the nine normal chromosomes in a set were identified using relative lengths and arm ratios, with the “missing” SC being identified as the SC with the inversion loop. For each of the eight inversion heterozygotes in which this test was possible, the identification of the inversion loop SC corresponded with the appropriate chromosome. This result confirms that our SC identifications are consistent with established linkage groups.

We examined 290 sets of SCs with RNs and were able to identify 2080 (~72%) individual SCs (Table 2, Figure 3). Some SCs were easier to identify than others, so the number of each SC analyzed for RNs varies. For example, SC 2, a long SC with an arm ratio near 1.0, and SC 10, the shortest SC, were relatively easy to identify, and as a result, we made 247 observations of each. In contrast, the number of observations for SC 6 and SC 7 was lower ( $n = 176$  and  $178$ , respectively) because they are similar in both relative length and arm ratio and more difficult to distinguish from one another. Nevertheless, these data represent the highest number of observations of RNs on individual SCs made for any organism except tomato (SHERMAN and STACK 1995).

**RN frequency per cell:** On the basis of the average frequency of RNs per SC, there is an average of 20.5 RNs per cell (Table 2). To verify that this number is representative, we examined 239 complete sets of SCs in which each SC could be analyzed for RN frequency,

even if not all of the SCs could be individually identified (usually because of the absence of discernible kinetochores). These complete sets of SCs averaged 20.7 RNs per SC set, a difference of <1% when compared to using RN averages for individual SCs. Thus, using RNs, we estimate that the total map length for maize inbred KYS is between ( $20.5 \text{ RNs} \times 50 \text{ cM/RN} =$ ) 1025 cM and ( $20.7 \text{ RNs} \times 50 \text{ cM/RN} =$ ) 1035 cM.

**Chiasma frequency per cell:** To compare rates of crossing over in KYS maize determined from RNs to those determined from chiasmata, we analyzed at least 50 squashes of chromosome sets at diakinesis from each of 5 plants (Figure 4) and at least 10 SC spreads from each of 10 plants (Table 3). No single plant was analyzed for both chiasmata and RNs. Each plant, regardless of the method of analysis, demonstrated large cell-to-cell variability (up to twofold) in the number of crossovers observed. While there were no significant differences among plants in variance for chiasmata or for RNs (Bartlett's test and Levene's test,  $P > 0.2$ ), there were significant differences among plants in the mean number of crossovers per cell on the basis of both chiasmata (ANOVA;  $P < 0.001$ ) and RNs (ANOVA;  $P < 0.001$ ). Because the plants were all from the same inbred strain and presumably had the same genetic makeup, we explored the possibility that environmental conditions were responsible for the differences in mean crossover frequency. The plants analyzed for chiasmata were all exposed to the same greenhouse conditions during the summer of 2002. The RN data were accumulated over

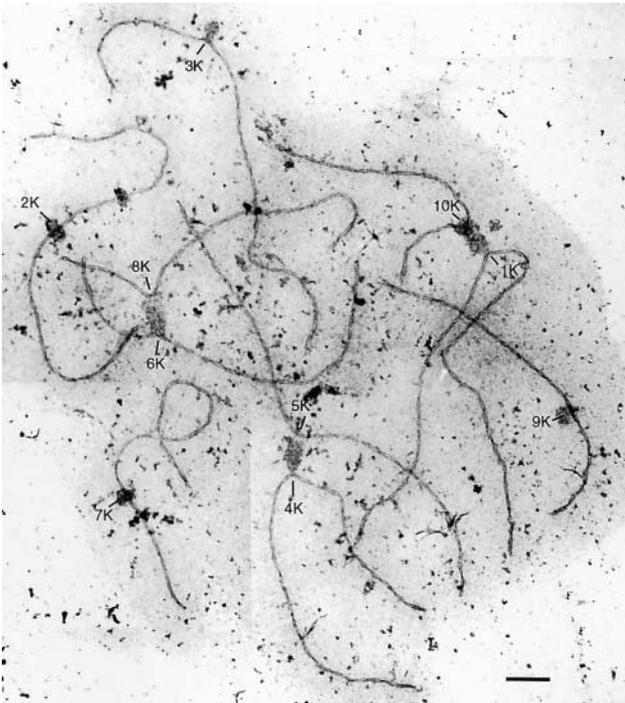


FIGURE 2.—Complete SC spread stained with uranyl acetate-lead citrate from a maize plant heterozygous for *In7a*. Each SC has been labeled at the kinetochore (K) with the appropriate SC number on the basis of its relative length and arm ratio. The inversion loop in the long arm of SC 7 can be seen in the lower left of the spread. Kinetochores from different SCs are often fused (6K and 8K, 1K and 10K, 4K and 5K). There are no obvious lateral element thickenings on any of the SCs. Bar, 5  $\mu$ m.

four years from 1998 to 2002, again using plants grown in the same greenhouse. SC spreads from 8 of the 10 plants were prepared in the summer (April–September) and SC spreads from 2 of the plants were prepared in the fall (October–March). The average number of RNs per cell was 19.8 and 21.5 for the 2 winter-grown plants and 17.0–23.1 for the 8 summer-grown plants. Although the data are limited, this pattern of RN numbers does not support the hypothesis that environmental factors are responsible for the differences in RN numbers observed.

Because the ranges of values for chiasmata and for RNs were similar among plants (even though there were significant differences in mean crossover frequency among plants), the data for chiasmata and RNs were pooled separately and compared. The average number of RNs per cell was  $\sim$ 10% higher (20.6) than the average number of chiasmata (18.9; Table 3). In addition to determining the average number of crossovers per cell, we also examined the frequencies of bivalents with 0, 1, 2, and 3 or more RNs and chiasmata (Table 3). The frequencies of bivalents with zero and one crossover per cell were the same whether RNs or chiasmata were used. However, the frequency of bivalents with two or more crossovers differed depending on the method of analysis

used. Bivalents with two crossovers were observed more often for chiasmata than for RNs, while bivalents with three or more crossovers were observed more often for RNs than for chiasmata. The difference in resolution of the two techniques may contribute to these observed discrepancies.

#### Relationship between RN frequency and SC length:

For 206 SC sets, we were able to determine both total RN number and total SC set length (although not all SCs could be identified in each spread; Figure 5A). The slope of the regression is significantly different from zero [total RNs =  $(0.026 \times \text{total SC length}) + 11.8$ ,  $P < 0.001$ ,  $r^2 = 16.3\%$ ]. Thus, SC set length and RN number per set are positively related, with  $\sim$ 16% of the variation in RN number explained by SC set length.

When the 10 maize bivalents are considered separately ( $n = 2080$  SCs, Tables 1 and 2), there is a strong positive relationship between average RN frequency and average SC length, with 96% of the variability in average RN frequency related to average SC length [Figure 5B;  $y = (0.042 \times \text{SC length}) + 0.66$ ,  $r^2 = 96.2\%$ ]. A similar relationship is observed when average SC arm lengths are compared to the average number of RNs per arm [Figure 5C,  $y = (0.044 \times \text{SC length}) + 0.31$ ,  $r^2 = 96.5\%$ ]. For both regressions, the slope and  $y$ -intercept are significantly different from zero ( $P < 0.001$ ). Thus, average RN frequency is closely correlated with individual SC length, whether one considers arm lengths separately or bivalent length as a whole.

**Distribution of RNs along SCs:** Histograms showing the distribution of RNs along each SC are presented in Figure 6. Each SC is represented by the  $x$ -axis with the short arm to the left and the kinetochore (kc) marked with a vertical line beneath the axis. Each SC is divided into 0.2- $\mu$ m segments with the number of RNs observed in each segment represented by a vertical bar. Two lines are superimposed over each distribution. One is a smoothing (Lowess) line derived from the data (CLEVELAND 1979), which shows the general trend of crossing over along each SC. The smoothing line minimizes variation regardless of whether the variation is caused by sampling errors or by localized differences in crossover frequency. The second superimposed line is horizontal and represents the average number of RNs observed per 0.2- $\mu$ m interval for each SC. Intervals that differ considerably from the horizontal lines represent hot (above average) or cold (below average) regions for crossing over.

All of the SCs share the general characteristics of a high frequency of RNs in distal regions (including the very ends of SCs) and a low frequency of RNs in proximal regions, *i.e.*, around kinetochores (Figures 6 and 7). The distributions of RNs on SCs 2, 4, and 9 indicate that a few RNs occur within kinetochores. This is an artifact due to compiling data from SCs that vary somewhat in arm ratios and plotting the distributions on an “average” SC. While RNs sometimes occur quite close

**TABLE 2**  
**Predicted map length based on the average number of RNs per SC compared to classical genetic and molecular (IBM) linkage maps**

SC no.	No. of SCs observed	SC		Genetic map <sup>a,b</sup>		IBM map <sup>a,c</sup>	
		Average no. of RN	Equivalent cM <sup>d</sup>	cM	Equivalent RN <sup>e</sup>	cM <sup>f</sup>	Equivalent RN <sup>e</sup>
1	216	2.69	134.3	258	5.2	325.3	6.5
2	247	2.37	118.4	224	4.5	204.2	4.1
3	189	2.22	110.9	216	4.3	228.4	4.6
4	196	2.14	107.1	172	3.4	228.3	4.6
5	203	2.21	110.6	185	3.7	202.6	4.1
6	176	1.81	90.6	144	2.9	164.2	3.3
7	178	1.86	93.0	128	2.6	188.7	3.8
8	194	1.80	90.2	177	3.5	189.2	3.8
9	234	1.85	92.7	178	3.6	193.9	3.9
10	247	1.54	76.9	174	3.5	157.4	3.1
Total	<b>2080</b>	<b>20.5</b>	<b>1024.7</b>	<b>1856</b>	<b>37.1</b>	<b>2082.2</b>	<b>41.8</b>

<sup>a</sup> From <http://www.agron.missouri.edu/cMapDB/cMap.html> (March 2003).

<sup>b</sup> Genetic map is the classical genetic map, compiled by Ed Coe from cooperators.

<sup>c</sup> IBM is an intermated B73/Mo17 recombinant inbred high-resolution molecular map.

<sup>d</sup> The average number of RNs  $\times$  50 cM/RN.

<sup>e</sup> Centimorgans divided by 50.

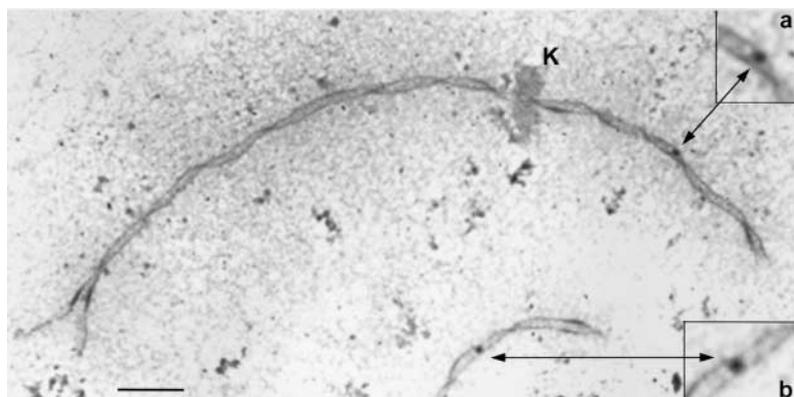
<sup>f</sup> This molecular map includes recombination events from three meioses (K. CONE, personal communication), so the centimorgans presented on the web site have been divided by 3 here for the purpose of comparison using the standard definition of centimorgans (TAMARIN 2002).

to the kinetochore, we did not observe any RNs that were clearly within kinetochores (Figure 7). In addition to the general trends in the distribution of crossing over that all SCs share, each SC also has a distinct pattern of RNs along its length.

To quantify the difference in RN frequency between distal and proximal segments, we divided each SC arm into five equal (20%) segments, pooled the total number of RNs observed for all of the most distal segments, and pooled the total number of RNs for all proximal segments. The combined distal segments for all SCs represent ( $0.20 \times 331 \mu\text{m}$  total SC length =)  $66.2 \mu\text{m}$  in length as do the combined proximal segments. If RNs were distributed evenly along the length of all SCs, then each 20% segment would have  $\sim 20\%$  of the total number of RNs observed. Instead, the most-distal SC

segments contain 48% (2061/4271) of all RNs observed while the most proximal segments contain 4% (156/4271) of all RNs observed. Thus, over the same SC length, distal regions have 12 times more crossing over than proximal regions do.

Maize KYS has five knobs, two on short arms (1S, 9S) and three on long arms (5L, 6L, 7L; DAWE *et al.* 1992; CHEN *et al.* 2000). The location of each knob is indicated by a horizontal line beneath the appropriate SC in Figure 6. Overall, the frequency of RNs in knob regions is either about the same as the average for the SC as a whole (5L, 6L, 7L) or higher (1S, 9S). This is particularly true for the knob on the tip of the short arm of SC 9, which has a frequency of RNs that is twice as high as the average for SC 9 (7 *vs.* 3.4 RNs per  $0.2\text{-}\mu\text{m}$  interval). In contrast, the NOR region on the short arm of SC 6



**FIGURE 3.**—Close-up of SC 9 from a set of SCs stained with uranyl acetate-lead citrate. A small portion of the distal end of the long arm is asynapsed, indicating that the SC has begun desynapsis and is in the earliest stage of diplotene. SC 9 has one RN (inset a) in the short arm. The dispersed chromatin around SC 9 is visible as an amorphous coating. A portion of SC 2 with an RN (inset b) is also shown. Double-headed arrows show the same RNs at higher magnification in insets. K, kinetochore. Bar,  $2 \mu\text{m}$  ( $1 \mu\text{m}$  in insets a and b).

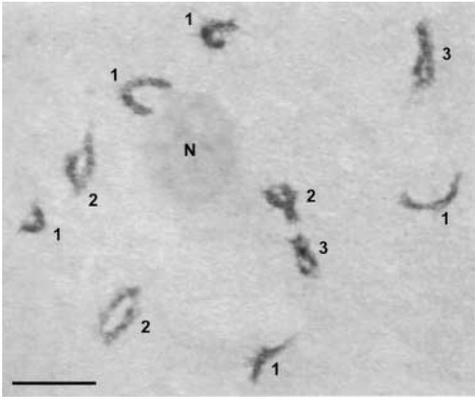


FIGURE 4.—Diakinesis chromosome squash from maize KYS. The counted number of chiasmata is indicated next to each bivalent. With the exception of chromosome 6 that is associated with the nucleolus (N), none of the chromosomes can be identified. Bar, 10  $\mu\text{m}$ .

has a slightly reduced level of RNs compared to the average for SC 6 (1.6 *vs.* 2.2 RNs per 0.2- $\mu\text{m}$  interval), but this lower level could be due to its proximity to the kinetochore (Figure 6).

#### DISCUSSION

**SC identification:** We have prepared a karyotype for SCs from KYS maize on the basis of relative lengths and arm ratios (Figure 1; Table 1). This SC karyotype is very similar to other maize pachytene karyotypes that have been prepared using a number of different techniques (aceto-carmin-stained pachytene chromosome squashes, 4',6-diamidino-2-phenylindole-stained intact pachytene nuclei, three-dimensional reconstructions of pachytene nuclei from serial thin sections, and SC spreads). The inbred KYS line of maize generally has been used in these studies because KYS pachytene chromosomes separate well during squashing and spreading, making them easier to analyze than pachytene chromosomes from many other maize lines (DEMPSEY 1994; our unpublished observations). In any case, there is little difference in the basic pachytene chromosome karyotype between

different maize lines, aside from the observation that the number and location of heterochromatic knobs may vary (LONGLEY 1939; McCLINTOCK *et al.* 1981; DEMPSEY 1994). However, while the presence of knobs can affect the length and arm ratios of mitotic chromosomes, the presence or absence of knobs has little effect on the relative length and arm ratios of SCs because heterochromatin is underrepresented in SC length (STACK 1984; JONES and DE AZKUE 1993). Thus, it is likely that the KYS SC karyotype applies in large measure to all maize lines.

Within animal and plant species (including maize) and even within individuals, there may be as much as a twofold variation in the absolute lengths of sets of pachytene chromosomes or SCs. However, the relative length and arm ratio for each chromosome or SC in a set remains constant (MOSES *et al.* 1977; GILLIES 1981; SHERMAN and STACK 1992; our observations). This indicates that each chromosome/SC in a set responds in a proportional way to changes in the length of the entire set.

Chromosome numbering in maize is based primarily on relative length with the longest chromosome numbered 1, ranging down to the shortest numbered 10. However, in each karyotype reported for maize, chromosome/SC 5 is slightly longer than chromosome/SC 4 (Table 1). This discontinuity in numbering arose because maize chromosomes were numbered initially using mitotic chromosomes that differ slightly in relative length from pachytene chromosomes (McCLINTOCK 1929; RHOADES 1955; CARLSON 1988). In addition, chromosome/SC 8 is longer than chromosome/SC 7 in some karyotypes but not in others (Table 1). These differences in relative length between different maize karyotypes are small and may represent either measurement error or natural variation within populations. In either case, these chromosomes are still readily distinguished from each other on the basis of differences in their arm ratios. In addition, we were able to verify the identity of SCs 1, 2, 3, 4, 5, 6, 7, and 9 using inversion heterozygotes. Although not verified by inversion heterozygotes, our identification of SCs 8 and 10 is also firm because both SC 8 and SC 10 can be readily distin-

TABLE 3

Comparison of crossover frequency for maize KYS using chiasmata and RNs

	No. of cells (bivalents) observed	Mean no. of RNs or chiasmata (SD) and range	No. of bivalents with 0–6 crossovers <sup>a</sup>				
			0	1	2	3	4–6
RNs	151	20.6 (3.3)	14	363	743	312	78
10 plants	(1510)	13–33	(0.9)	(24.0)	(49.2)	(20.7)	(5.2)
Chiasmata	278	18.9 (2.5)	31	667	1673	402	7
5 plants	(2780)	12–26	(1.1)	(24.0)	(60.2)	(14.5)	(0.3)
<i>P</i> values <sup>b</sup>		<0.001	0.56	0.97	<0.001	<0.001	<0.001

<sup>a</sup> Numbers in parentheses represent percentages.

<sup>b</sup> Student's *t*-test using mean frequency of bivalent type per cell.

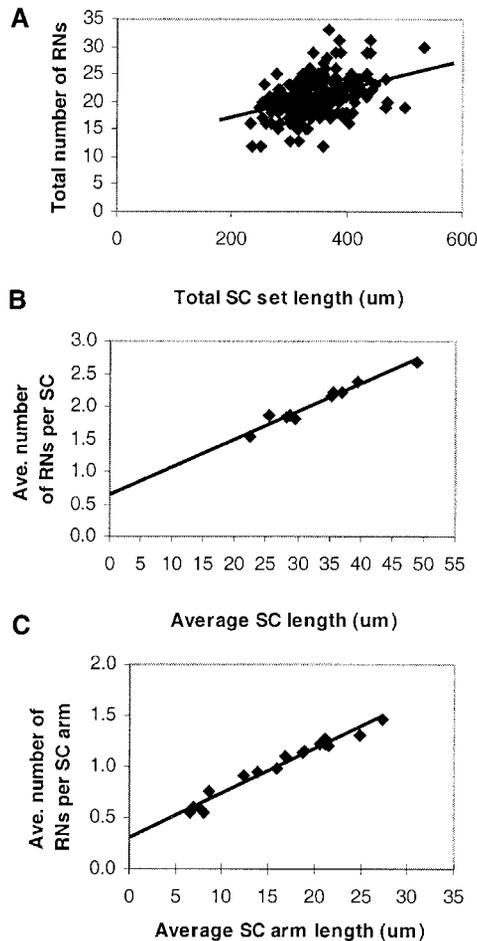


FIGURE 5.—Scatter plots and regressions for (A) total SC set length and total RN number ( $y = 0.026x + 11.82$ ,  $r^2 = 0.16$ ), (B) average SC length and average RN frequency ( $y = 0.042x + 0.66$ ,  $r^2 = 0.96$ ), and (C) average SC arm length and average RN frequency ( $y = 0.044x + 0.31$ ,  $r^2 = 0.97$ ).

guished from the other SCs and from each other on the basis of their relative lengths and arm ratios.

The most striking difference among maize karyotypes involves the arm ratio for chromosome/SC 6. The average arm ratio is 3.0 or higher for squashed pachytene chromosomes and sectioned SCs, but only 2.6–2.7 for SC spreads (Table 1). Since the short arm of chromosome/SC 6 carries the NOR, it is likely that differences between the karyotypes somehow involve the nucleolus. Typically, a single large nucleolus is visible in primary microsporocytes that are fixed before squashing or sectioning (RHOADES 1950; DAWE *et al.* 1994). In contrast, SC spreads are prepared by exposing live cells to a hypotonic solution in which nucleoli and chromatin are dispersed before fixation. The larger arm ratio of SC 6 in squashes compared to SC spreads could be due to relative shortening of the NOR short arm in squashes (perhaps because the nucleolus obscures part of the arm) or lengthening of the short arm in SC spreads. In this regard, SHERMAN and STACK (1992) observed that the short arm of SC 2 in tomato was often broken at the

location of the NOR in spreads. However, we did not observe any unusual fragmentation or any detectable stretching of the SC in the short arm of maize SC 6. Whatever the cause of the differences in arm ratio for chromosome/SC 6, we are confident that we can identify SC 6 because the arm ratio for SC 6 is consistent between sets of SC spreads, SC 6 can be separated reliably from the other SCs, and SC 6 has been verified to correspond to chromosome 6 by analysis of SC spreads from inversion heterozygotes for *In6b*.

**Estimating crossover frequency using different methods:** Some controversy surrounds estimates of crossover frequency that have been determined using chiasmata, RNs, and linkage maps (*e.g.*, NILSSON *et al.* 1993; SHERMAN and STACK 1995; SYBENGA 1996; KING *et al.* 2002a). Typically, saturated or nearly saturated classical linkage maps indicate higher crossover rates than chiasma counts, and molecular linkage maps often indicate even higher crossover rates (*e.g.*, NILSSON *et al.* 1993; MORAN *et al.* 2001; KING *et al.* 2002a; Table 3). This also appears to be the pattern in maize where chiasma counts range from an average of 17–21 per cell when diakinesis-metaphase I chromosomes are used (BEADLE 1933; AYONOADU and REES 1968; PAGLIARINI *et al.* 1986; Table 3) although higher averages (27–37 per cell) are possible when longer diplotene chromosomes are used (in which chiasmata are more difficult to distinguish from twists; BEADLE 1933; DARLINGTON 1934). In comparison, genetic and molecular linkage maps indicate 37 and 42 crossovers per cell, respectively (Table 2). This problem has been addressed recently by KING *et al.* (2002a), who introgressed a single chromosome of *Festuca pratensis* into *Lolium perenne* to create a monosomic substitution line (hereafter referred to as the *Festuca/Lolium* bivalent). The *F. pratensis* chromosome synapses and recombines with the homeologous *L. perenne* chromosome, although the two chromosomes can be distinguished from one another using genomic *in situ* hybridization. KING *et al.* (2002a) compared the recombination frequency for this homeologous bivalent using both amplified fragment length polymorphism markers and chiasmata. They found a 1:1 relationship between crossovers detected by the two methods. They suggest that chiasma counts tend to somewhat underestimate the amount of crossing over due to difficulty in resolving nearby chiasmata, while molecular maps tend to inflate the amount of crossing over due to errors in typing and the use of different computer programs with different algorithms to generate the maps. KING *et al.* (2002a) also pointed out that differences in map lengths are not surprising, given that various investigators employed a variety of mapping techniques on different mapping populations. Recently, KNOX and ELLIS (2002) showed in pea (*Pisum sativum*) that excess heterozygosity in the mapping population can also lead to inflation of the molecular map compared to chiasma counts. They conclude that a number of factors contribute to map inflation when using

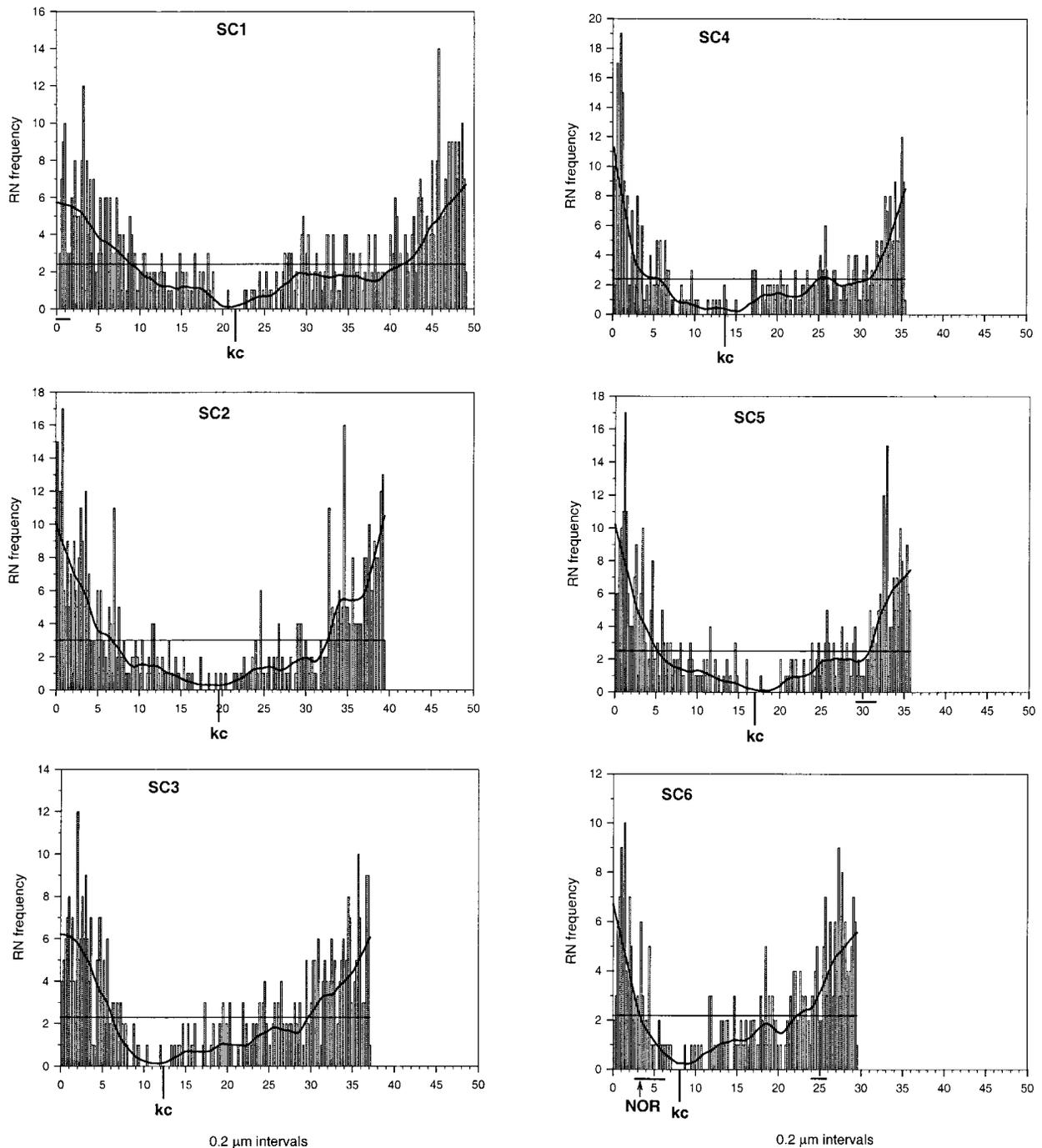


FIGURE 6.—Histograms showing the distribution of RNs along the length of each maize SC. Each SC is represented on the  $x$ -axis with the short arm to the left and the position of the kinetochore ( $kc$ ) marked with a vertical line. The positions of the NOR (6S) and knobs (1S, 5L, 6L, 7L, 9S) are indicated as short horizontal bars beneath the  $x$ -axis. The histogram bars represent the total observed number of RNs in each 0.2- $\mu$ m SC length interval. Superimposed over each distribution is a thick smoothing line that shows the general trend of the RN distribution as well as a thinner horizontal line that represents the average number of RNs in each 0.2- $\mu$ m interval.

molecular markers and that chiasmata and RNs yield good estimates of crossover frequency.

Here, we show that in KYS maize the frequency of chiasmata compares well with the frequency of RNs, particularly when the frequency of homologous pairs with zero or one chiasma is compared with the frequency of SCs with

zero or one RN (Table 3). When higher categories of crossing over are compared, the number of bivalents with two chiasmata is higher than the number of bivalents with two RNs, while the number of bivalents with three or more chiasmata is lower than the number of bivalents with three or more RNs. Given the difference

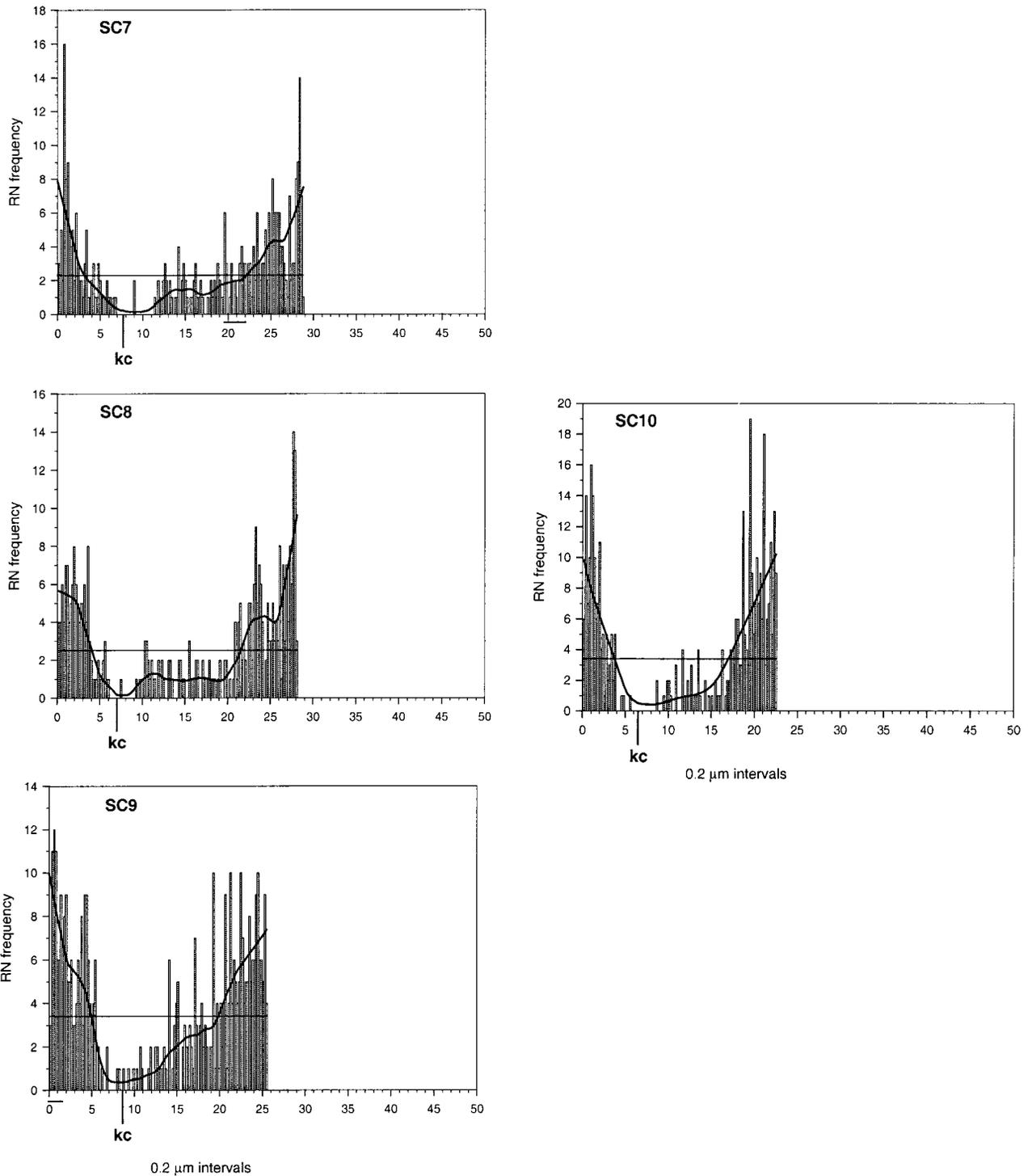


FIGURE 6.—Continued.

in resolution between RNs and chiasmata (STACK *et al.* 1989; SHERMAN and STACK 1995), it is likely that multiple crossovers are more difficult to identify using chiasmata than using RNs, and some of the bivalents classified with two chiasmata probably had three or more. Overall, we estimate that our counts of chiasmata in maize underestimate the amount of crossing over by ~10% compared to our estimates of crossing over based on RNs.

#### Inbred KYS and the rate of crossing over in maize:

Is the rate of crossing over in inbred KYS representative of the rate of crossing over for maize in general? This question is of importance because KYS has long been used as the favorite inbred line for the study of maize chromosomes, but genetic maps and molecular maps typically use data from different lines and from crosses that do not include KYS (<http://www.agron.missouri>).

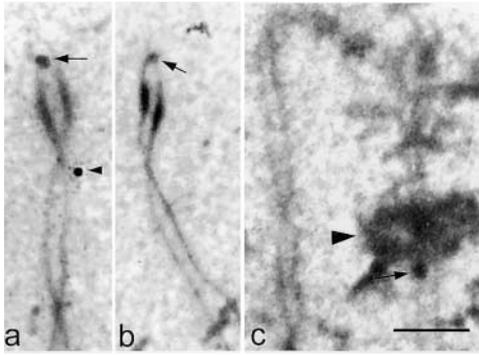


FIGURE 7.—Portions of three maize SCs stained with uranyl acetate-lead citrate. (a, b) RNs can occur at the very distal tip of an SC (arrows). Stain precipitate (small arrowhead) is much darker than RNs and has sharp edges. Thickenings of lateral elements are present in a and b. (c) Rarely, an RN (arrow) can be observed close to the kinetochore (large arrowhead). Bar, 1  $\mu$ m.

edu/maps.html). Since no other maize line has been analyzed for RNs, comparisons of RN numbers in different lines currently are not possible. However, as an alternative, we compared chiasma frequencies between KYS and three other commonly used inbred lines (W22, B73, Mo17) and a B73/Mo17 hybrid (the cross used to prepare the IBM map, an intermated B73/Mo17 recombinant inbred high-resolution molecular map). We found a 12% difference in average number of chiasmata per cell between lines with the least (Mo17) and the most chiasmata (W22) with the other lines (KYS, B73, and the B73/Mo17 hybrid) falling in between (Table 4). Our results are consistent with those of a number of investigators who have found differences in recombination frequency among inbred lines and crosses for maize (*e.g.*, PAGLIARINI *et al.* 1986; BEAVIS and GRANT 1991; FATMI *et al.* 1993; WILLIAMS *et al.* 1995; TIMMER-

MANS *et al.* 1997), rye grass (KARP and JONES 1982), pea (HALL *et al.* 1997), and mouse (KOEHLER *et al.* 2002). Because the average difference in chiasma frequency that we observed between different lines in maize are minor (only 1–2 per cell), it is likely that all of the lines would have overall RN distributions similar to KYS. On the other hand, it is possible that there could be significant differences if one examines only a small portion of any particular SC, particularly in proximal regions that have few RNs and where the addition or subtraction of only a few RNs would have larger effects than in distal regions where most RNs were observed.

**The frequency of RNs and SC length:** We found a positive correlation between total SC set length and the total number of RNs per SC set in maize with ~16% of the variation in RN number explained by variations in SC set length ( $r^2 = 0.16$ ; Figure 5). Similar relationships have been reported for humans and certain strains of mice ( $r^2 = 0.13$ – $0.33$ ; LYNN *et al.* 2002), and reanalysis of the data from SHERMAN and STACK (1995) indicates that a similar relationship also holds in tomato ( $r^2 = 0.13$ ). However, there is only a weak relationship between SC set length and number of MLH1 foci for males of the mouse inbred strain C57BL/6 ( $r^2 = 0.04$ ), although females of this strain do show a positive relationship (FROENICKE *et al.* 2002; LYNN *et al.* 2002). LYNN *et al.* (2002) showed that the pachytene substage did not affect SC set length for humans, and similar  $r^2$  values (13–16%) for other species suggest that this might be true for these species as well. However, it has been frequently (but not always) noted that SC sets are longer in early pachytene than in late pachytene (*e.g.*, MOSES *et al.* 1977; MAGUIRE 1978; GILLIES 1981). Since it is likely that the number of RNs does not change during pachytene (SHERMAN and STACK 1995), any variation in SC length due to pachytene substage could obscure

TABLE 4

Comparison of the mean number of chiasmata for four different inbred lines and a hybrid between two inbred lines (B73/Mo17)

Inbred line	No. of cells (bivalents)	Mean no. of chiasmata (SD)	No. of bivalents with <sup>a</sup>			
			0 chiasmata	1 chiasmata	2 chiasmata	$\geq 3$ chiasmata
W22	267 (2670)	21.2 (1.9)	2 (0.1)	303 (11.3)	1758 (65.8)	607 (22.7)
B73/Mo17	267 (2670)	21.0 (1.8)	1 (0.0)	267 (10.0)	1892 (70.9)	510 (19.1)
B73	270 (2700)	20.9 (2.2)	10 (0.4)	355 (13.1)	1753 (64.9)	582 (21.6)
KYS	278 (2780)	18.9 (2.5)	31 (1.1)	667 (24.0)	1673 (60.2)	409 (14.7)
Mo17	283 (2830)	18.5 (1.8)	15 (0.5)	607 (21.4)	1983 (70.1)	225 (8.0)

Five plants from each line were sampled. The mean number of chiasmata varies significantly between lines (ANOVA,  $P < 0.001$ ) as do the variances (Levene's  $P < 0.001$ ).

<sup>a</sup> Numbers in parentheses represent percentages.

TABLE 5

Comparison of SC length from spreads, number of crossovers (based on RNs or MLH1 foci), and number of bivalent arms for male meiotic cells from mouse, humans, tomato, and maize

Species	Genome size (1C pg)	Average SC set length ( $\mu\text{m}$ )	Average no. crossovers per set	No. of SCs	No. of SC arms	References
Mouse	3.3	148–170	21.5–24.9	19	19	GREGORY (2001); FROENICKE <i>et al.</i> (2002); KOEHLER <i>et al.</i> (2002)
Human	3.5	290	49.1	22	44	GREGORY (2001); LYNN <i>et al.</i> (2002)
Chicken	1.25	203	65.0	38	41	RAHN and SOLARI (1986); GREGORY (2001); PIGOZZI (2001)
Tomato	1.03	229	21.9	12	23 <sup>a</sup>	SHERMAN and STACK (1995); BENNETT <i>et al.</i> (2000)
Maize	2.73	331	20.5	10	20	BENNETT <i>et al.</i> (2000); this work
Lily	35.2	3149	55	12	14	ANDERSON <i>et al.</i> (1985); STACK <i>et al.</i> (1989); BENNETT <i>et al.</i> (2000)

<sup>a</sup> The short arm of chromosome 2 is totally heterochromatic so this arm is excluded here.

the relationship between SC set length and the number of RNs. With this caveat, these data suggest that variation in total SC set length within a species is related to variation in the total amount of crossing over. Also supporting this conclusion is the observation that some female animals have more crossing over than male animals (*e.g.*, zebrafish: SINGER *et al.* 2002) as well as longer total SC set lengths (humans: BOJKO 1985; WALLACE and HULTÉN 1985; SPEED and CHANDLEY 1988; TEASE *et al.* 2002).

Does the relationship between SC length and RN frequency hold between species; *i.e.*, do species with longer total SC lengths necessarily have more RNs? The answer appears to be no (Table 5). Even though SC length is closely correlated with genome size (flowering plants: ANDERSON *et al.* 1985; bony fish, reptiles, mammals, but not birds: PETERSON *et al.* 1994) and genome size varies greatly among eukaryotes, the number of coding genes is relatively constant. Since most crossing over takes place within genes, the frequency of crossing over between species is better correlated with the number of genes than with total genome size (THURIAUX 1977 and many subsequent reports; Table 5) or with total SC length. Then why are there differences in the rates of crossing over between eukaryotic species? First, species with larger numbers of chromosomes will have more crossing over than species with lower numbers of chromosomes due to the obligate crossover between each homologous pair (*e.g.*, chicken, Table 5). Second, the number of chromosome arms is also positively correlated with crossover frequency with an average of one crossover per arm for mammals (PARDO-MANUEL DE VILLENA and SAPIENZA 2001). Tomato and maize fit this general trend as well (SHERMAN and STACK 1995; this work). However, both tomato and maize have (short) chromosome arms that average less than one RN as well as (long) arms that average more than one RN (Figure 5; SHERMAN and STACK 1995). Third, species with excep-

tionally large genomes and chromosomes have long SCs and more crossing over (although the relation is not proportional), possibly because crossover interference attenuates enough on long SCs to permit additional crossover events (see lily, Table 5). Finally, the strength of crossover interference may differ from one group to another; *e.g.*, budding yeast with 16 short SCs has ~100 crossovers (PÂQUES and HABER 1999). Thus, while it is clear that crossing over is tightly regulated, it is equally clear that a number of factors may be involved in this regulation and that not all factors may operate in the same way in different species (ANDERSON and STACK 2002).

How is the rate of crossing over controlled for individual bivalents in a set? It has been recognized for some time that relative chromosome length is positively correlated with the level of crossing over and chiasma formation within a species (*e.g.*, MULLER 1916; DARLINGTON 1934; MATHER 1937). More recently, in-depth studies have been done on recombination frequency in tomato, chicken, mouse, and maize in which individual bivalents were identified and examined for RNs or MLH1 foci. All four studies revealed a strong positive correlation between average SC length and average RN frequency for each bivalent ( $r^2 \geq 0.96$  for each species excluding the three shortest SCs from mouse; SHERMAN and STACK 1995; PIGOZZI 2001; FROENICKE *et al.* 2002; this study). The rarity of SCs without RNs in maize and tomato (SHERMAN and STACK 1995) and the rarity of pachytene bivalents without MLH1 foci in mice and chicken (PIGOZZI 2001; FROENICKE *et al.* 2002) illustrate the tight control of crossing over that ensures each bivalent (but not necessarily each arm) has at least one crossover while at the same time apportioning the probability of additional crossovers according to SC length. Thus, there appear to be at least two levels of control for crossover frequency, one at the level of the cell and another at the level of individual bivalents and bivalent arms.

**The distribution and frequency of crossing over along maize SCs:** Generally, all 10 maize SCs show similar patterns of RN distribution (Figure 6). The distal portions of the arms invariably have the highest average concentration of RNs, while the frequency of RNs trails off proximally toward the kinetochore, where RNs are absent. This RN pattern is expected from reports of a high distal concentration of chiasmata in maize (Figure 4; RHOADES 1950, 1955). This RN pattern also is similar to high levels of distal crossing over that have been reported for two other grass species, wheat (GILL *et al.* 1993, 1996a,b) and barley (KÜNZEL *et al.* 2000). In addition to the general concentration of RNs distally in maize, there are distinct peaks of RNs involving one or a few adjacent 0.2- $\mu$ m SC segments. Distinct chromosomal regions of higher recombinational activity have been reported also for barley (KÜNZEL *et al.* 2000) and wheat (GILL *et al.* 1996a). These “hot” regions for crossing over at the chromosomal level (FROENICKE *et al.* 2002) may correspond to hotspots of recombination at the DNA level, such as the well-known hotspots at *al* and *bz1* loci that occur in distal regions of maize chromosomes 3 and 9, respectively (BROWN and SUNDARESAN 1997; FU *et al.* 2001, 2002; YAO *et al.* 2002). However, such peaks (as well as valleys) in the histograms also may be caused (or exacerbated) by compiling data on the location of RNs from many different SCs onto an “average” SC or by sampling errors. The smoothing lines reduce such variation and show the general trends of crossing over along the SCs. Determining whether the individual peaks/valleys are artifactual or represent definite positions of elevated or reduced recombination frequency will require additional study. One method to address this question will be to use fluorescence *in situ* hybridization to determine the precise locations of mapped loci that are known to be hot or cold spots for crossing over (HARPER and CANDE 2000; SADDER *et al.* 2000; SADDER and WEBER 2002).

Aside from the immediate vicinity of the kinetochore, no large segment on any of the maize SCs is completely free of RNs, but there are segments proximal to kinetochores on every SC in which there are only a few RNs (Figures 6 and 7). A low level of recombination in and near centromeres also has been detected by molecular mapping in *Arabidopsis* (COPENHAVER *et al.* 1998) and in the *Festuca/Lolium* bivalent (KING *et al.* 2002b). A reduced level of crossing over proximally is probably related to the centromere effect (RESNICK 1987) and to the presence of pericentric heterochromatin in the arms of all maize SCs (CARLSON 1988; JEWELL and ISLAM-FARIDI 1994). Pericentric heterochromatin in maize does not stain by C-banding, so it may be a “lower grade” of heterochromatin that permits more crossing over nearer the centromere rather than the dense blocks of pericentric heterochromatin in tomato (SHERMAN and STACK 1995). Even so, the pericentric regions of maize chromosomes are potential sites for blocks of genes that

are protected from recombination, possibly including the genes that differentiate maize from teosinte (GALINAT 1988; DOEBLEY 1994).

RNs in maize can occur at the very ends of SC arms (Figures 6 and 7), in contrast to tomato in which no RNs ( $n = 9058$  observations) were at the ends of SCs (SHERMAN and STACK 1995). Inhibition of crossing over at the ends of tomato chromosomes is probably related to the presence of prominent dark-staining telomeres with associated repeated DNA sequences that may have some properties of heterochromatin (SHERMAN and STACK 1995; ZHONG *et al.* 1998). In maize, telomere structures are not obvious, suggesting that maize telomeres are small and do not interfere with nearby crossing over.

**The effect of knobs and NORs on crossing over:** Heterochromatic knobs are a characteristic feature of maize pachytene chromosomes with the exact number and placement of knobs varying between different lines (McCLINTOCK *et al.* 1981). Knobs are composed of large tandem arrays of 180- and 350-bp repeats (PEACOCK *et al.* 1981; ANANIEV *et al.* 1998; CHEN *et al.* 2000). Because knobs are heterochromatic, they usually are considered to be recombinationally inert, although variation in the sizes of knobs within populations could be explained by unequal crossing over within the knobs (BUCKLER *et al.* 1999). While we were unable to observe knobs due to dispersion of chromatin in SC spreads, we estimated the position of homozygous knobs on KYS maize SCs using data from DAWE *et al.* (1992) and CHEN *et al.* (2000). We found that the amount of crossing over at the location of knobs was either about the same as the average for the SC as a whole (interstitial knobs 5L, 6L, 7L) or higher than the average (distal knobs 1S and 9S; Figure 6). The knobs on 1S, 9S, and 6L are comparatively small, so they might not be expected to have much effect on RN frequency. However, the knobs on 5L and 7L are large, and yet they still have appreciable numbers of RNs. This result indicates that knobs have little or no effect on reducing the amount of crossing over at their locations on SCs. However, since knobs are heterochromatic, the structural relationship of knobs to SC may differ from the structural relationship of euchromatin to SC. The generally accepted model for SC structure has loops of DNA (chromatin) extending from each lateral element that may include a cohesin core (ZICKLER and KLECKNER 1999; VAN HEEMST and HEYTING 2000; PELTTARRI *et al.* 2001; STACK and ANDERSON 2001; EIJPE 2002). We suggest that the DNA in knobs is in the form of one to a few long loops of condensed chromatin that are anchored to a short region of the lateral elements (Figure 8). Knobs, and heterochromatin in general, may have only a few anchoring sequences in comparison to euchromatic regions. This model agrees with the explanation offered by STACK (1984) for the observed underrepresentation of heterochromatin in the length of pachytene chromosomes. In addition, it is supported by the demonstration that chromatin loops are

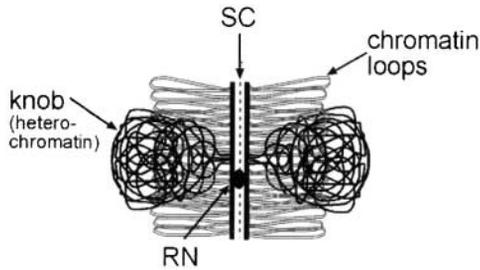


FIGURE 8.—An SC with euchromatic chromatin loops and a homozygous heterochromatic knob. Numerous (shaded) loops of euchromatin extend from the cohesin/lateral element core from regularly spaced attachment sites. The loops are shown doubled to represent the two sister chromatids, but sister loops may actually extend in opposite directions (STACK and ANDERSON 2001). In contrast, knob heterochromatin may consist of only a few (solid) loops and their attachment sites. Since crossing over occurs within the context of the SC, the reduced association of the knob chromatin with the SC would greatly decrease, but not eliminate, the amount of crossing over that could occur within the knob itself. However, as shown here, RNs that appear to be within the region of the knob could actually be mediating crossovers between euchromatic loops. This would explain why RN frequencies at heterochromatic knobs appear to be about average (or above) for the SC region where the knobs occur.

longer in pericentric heterochromatin than in distal euchromatin along tomato SCs (PETERSON *et al.* 1996). Since crossing over in plants and animals occurs in the context of the SC, minimal association of knob chromatin with the SC could sharply reduce crossing over within homozygous knobs. This model would explain why homozygous knobs neither alter the local rate of recombination nor add significantly to the length of pachytene chromosomes (RHOADES 1955; McCLINTOCK *et al.* 1981; STACK 1984; JONES and DE AZKUE 1993).

The frequency of RNs in the NOR region in the short arm of chromosome 6 is slightly lower than that for the SC as a whole (1.6 RNs *vs.* 2.2 RNs per 0.2- $\mu$ m segment; Figure 6). Crossing over within the NOR also has been detected in the *Festuca/Lolium* bivalent although at a reduced level compared to other parts of the chromosome (KING *et al.* 2002b). Considering that NORs are made up of tens to thousands of tandem repeats (HESLOP-HARRISON 2000), NOR DNA may have few anchor sequences for SCs and a relation to SC similar to that proposed for knobs and heterochromatin in general. As a result, crossing over within the NOR itself would be lower while having little effect on the rate of recombination in nearby regions.

**RN maps compared to linkage maps:** Two linked genes that recombine 1% of the time during a single meiosis are separated by 1 map unit (centimorgan), and 50 map units correspond to the map distance between two loci in which there is an average of one crossover event per meiosis. Since each RN corresponds to a crossover event, an SC segment that averages one RN per meiosis would also be 50 map units long. Thus, the average of 20.5

RNs per meiosis for a complete set of KYS maize SCs (Table 2) is equivalent to a total map length of 1025 cM. How do the lengths of the RN maps compare with the classical gene maps and the molecular linkage maps for maize? From Table 2, it is apparent that the genetic and the molecular linkage maps are both roughly twice as long as the RN map. However, when pairwise comparisons are made between map lengths of individual chromosomes (linkage groups) using different maps, all maps are significantly correlated ( $P < 0.01$ ). The predictive value of the correlation is best for the RN map compared to the molecular map ( $r^2 = 76\%$ ), with lower values for the RN map and the gene map ( $r^2 = 63\%$ ) and the gene and molecular maps ( $r^2 = 59\%$ ). These correlations are in the same order but better than the same correlations reported for tomato ( $r^2 = 69, 45,$  and  $21\%$ , respectively; SHERMAN and STACK 1995). Thus, differences in crossover frequency between the maps for maize seem to apply to all 10 bivalents more or less equally.

The shorter length of the RN map compared to the linkage maps could be explained if some RNs are lost (perhaps due to the spreading technique or to RN turnover), and, indeed, a small number of maize SCs are without RNs (Table 3). However, this is an unlikely explanation for three reasons:

1. The number of univalent pairs from diakinesis chromosome squashes matches the number of maize SCs without RNs ( $= 0.1\%$ ), so this level of failure to cross over appears to be a normal feature of crossing over in maize whether measured at pachytene or diakinesis (Table 3).
2. The difference in size between RN maps and linkage maps requires that about half the RNs would have to be lost so that each SC would average two RNs (as actually observed) instead of the “real” four RNs predicted from linkage maps. If RNs were lost at random and each RN had a 50% chance of being lost, then one would expect  $\sim 6\%$  ( $= 1/2^4$ ) of the SCs to have no RNs. This is 60 times more SCs with no RNs than were actually observed.
3. Finally, the RN map is slightly larger than the chiasma map (Table 3), so if we are losing half the RNs, we must likewise be counting less than half the chiasmata, which again seems unlikely.

So which of the maps most accurately describes the amount of crossing over in maize? We argue that the RN map is the most accurate (at least for male KYS) for several reasons:

1. The close agreement between numbers of RNs and chiasmata is independent support for the accuracy of the RN map (Table 3).
2. The RN map was prepared using a single inbred line, whereas linkage maps were prepared using a variety of lines or hybrids.

3. The RN map is based only on male meiosis, whereas linkage maps utilize the products of both male and female meioses that may differ in rate and distributions of crossing over (RHOADES 1941, 1978; CARLSON 1988).
4. The conditions under which the work was performed favors the consistency of the RN map because the RN map was produced from plants grown under the same conditions by the same people using the same instruments and techniques.
5. Several factors have been identified that could lead to inflated linkage map values (LINCOLN and LANDER 1992; KING *et al.* 2002a; KNOX and ELLIS 2002).
6. Since crossing over takes place in the context of pachytene chromosomes, it is noteworthy that RN map lengths are better correlated with individual SC lengths than with either genetic or molecular linkage maps. The lower correlations with the linkage maps may reflect uneven coverage of markers among the chromosomes.

**Uses of RN maps:** RN maps show the physical distribution of crossing over along each bivalent. Such maps can be used in a variety of ways. For example, they can be used to compare gene evolution in regions of the chromosome with high and low RN frequency (STEPHAN and LANGLEY 1998; TENAILLON *et al.* 2002), to examine interference (SHERMAN and STACK 1995; ANDERSON *et al.* 1999; FROENICKE *et al.* 2002; our unpublished results), and to aid in integrating linkage maps with chromosome structure (PETERSON *et al.* 1999; FROENICKE *et al.* 2002; our unpublished results).

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