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Crossing over as assessed by late recombination nodules is related to the pattern of synapsis and the distribution of early recombination nodules in maize

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Abstract

Recombination nodules (RNs) are multicomponent proteinaceous ellipsoids found in association with the synaptonemal complex (SC) during prophase I of meiosis. Numerous early RNs (ENs) are observed during zygotene, and they may be involved in homologous synapsis and early events in recombination. Fewer late RNs (LNs) are observed during pachytene, and they occur at crossover sites. Here we describe the pattern of synapsis and the distribution of ENs and LNs in maize. Synapsis starts almost exclusively at chromosome ends, although later in zygotene there are many interstitial sites of synaptic initiation. ENs do not show interference, except possibly at distances ≤ 0.2 μm. The frequency of ENs is higher on distal compared to medial SC segments, and the highest concentration of ENs occurs at synaptic forks. The number of ENs on an SC segment does not change during zygotene. These observations are interpreted to indicate that ENs are assembled at synaptic forks. Like ENs, LNs are more concentrated distally on bivalents but, unlike ENs, LNs show interference. A model is presented that relates the pattern of synapsis and ENs to the pattern of late nodules and crossing over.

Introduction

Chromosomes align, synapse and cross over during early prophase I of meiosis. Some or all of these events may be mediated by multicomponent proteinaceous ellipsoids called recombination nodules (RNs) that range from 50 to 200 nm in their longest dimension. RNs are found in association with both axial elements (AEs) and synaptonemal complexes (SCs) from leptotene through pachytene (Carpenter 1975, Anderson *et al.* 2001, and see von Wettstein *et al.* 1984, Stack *et al.* 1993, Roeder 1997, Zickler and Kleckner 1999 for reviews). Two types of RNs are recognized, early nodules (ENs) and late nodules (LNs) that are differentiated on the basis of relative numbers, size, shape, and staining characteristics (Stack & Anderson 1986a, Albini and Jones 1987, 1988, Carpenter 1987, 1988, Sherman *et al.* 1992, Zickler & Kleckner 1999).

LNs are found during pachytene at sites along SCs where crossing over occurs and where chiasmata will form later. Indeed, LNs probably represent the molecular factories that accomplish crossing over (e.g. Carpenter 1975, 1979, Albini & Jones 1984, Bernelot-Moens & Moens 1986, Sherman & Stack 1995, Stack & Roelofs 1996). On the other hand, the role of ENs is less clear. In comparison with LNs, ENs are more variable in size, are found in larger numbers (2–20× as many per unit length of SC), and occur more or less randomly along axial elements (AEs) and SCs during leptotene and zygotene (Rasmussen & Holm 1980, Stack & Anderson 1986a, 1986b, Albini & Jones 1987, Anderson *et al.* 2001). Because ENs are often found where axial elements converge prior to synapsis, they may have a role in recognition of homologs and initiation of SC formation (e.g. Albini & Jones 1987, Anderson & Stack 1988, Rockmill *et al.* 1995). Also the recA-related proteins, Rad51p and Dmc1p, that are known to be involved in recombination have been localized to ENs so it is probable that ENs also have a role in recombination (Anderson *et al.* 1997, Moens *et al.* 1997, Tarsounas *et al.* 1999). The relationship of ENs to LNs is not clear, but differential silver staining (Sherman *et al.* 1992) and immunolocalization of proteins involved in recombination suggest that a subset of ENs become LNs (von Wettstein *et al.* 1984, Stack & Anderson 1986b, Plug *et al.* 1998, Zickler & Kleckner 1999, Agarwal & Roeder 2000, Ashley & Plug 1998, Novak *et al.* 2001).

Although ENs appear to be involved in recognition of homologs and recombination, there have been relatively few studies of the distribution of ENs along SCs (Rasmussen & Holm 1978, Carpenter 1979, Holm *et al.* 1981, Holm & Rasmussen 1983, Stack & Anderson 1986b, Albini & Jones 1987, Anderson *et al.* 2001), and there have been only two reports of what may be ENs on SCs in cereal grains (wheat – Hobolth 1981; rye – Abirached-Darmency *et al.* 1983). As the most economically important plants, cereal grains have been and continue to be intensively studied from both chromosomal and genomic perspectives (e.g., Freeling & Walbot 1994). In an effort to more thoroughly understand the factors involved in the control of crossover number and distribution in this group of plants, here we describe the pattern of synapsis and the quantitative distribution of ENs and LNs in *Zea mays* (maize), the classic model grass species for cytogenetic studies of pachytene chromosomes and recombination (e.g., McClintock 1931, 1934, 1941, Rhoades 1955, Burnham 1980).

Materials and methods

Seeds from maize inbred strain KYS and seeds that were heterozygous for an inversion in chromosome 7 (*In7a*) in KYS background were grown to flowering in a temperature-controlled greenhouse at about 25ºC. Aceto–orcein-stained squashes of primary microsporocytes from anthers of different lengths revealed that anthers from 1.6–2.0 mm in length usually contain cells at zygotene and anthers from 2.1–2.2 mm in length usually contain cells in pachytene. Zygotene SC spreads used in this study were obtained from one KYS plant and one *In7a* plant. Pachytene SC spreads were obtained from four KYS plants.

To prepare spreads of SCs, 30–60 anthers were placed in a depression slide containing 0.2 ml of digestion medium that was 0.1 mmol/L Piperazine-N,N′-bis[2-ethanesulfonic acid] (PIPES), 0.5 mmol/L KH_2PO_4, 0.5 mmol/L $CaCl_2$, 0.2% potassium dextran sulfate (Calbiochem), 1.0% polyvinlypyrrolidone (PVP), and 0.7 mol/L mannitol at pH 4.1. Using a microscalpel, the anthers were cut in half transversely to their long axis, and then 3 mg of desalted pectinase (Sigma) was added. After digestion in a dark humid chamber for 20–40 min at 20ºC, 3 mg of desalted cytohelicase (Sepracor) was added. After 20 min of further digestion, primary microsporocyte protoplasts were squeezed out of the anthers with steel needles. The protoplasts were picked up in a volume of about 0.3 μl of the digestion medium using a micropipet. This suspension was put into 10 μl of bursting medium (0.05% Igepal CA-630, Sigma, a non-ionic detergent, and 1% BSA) at the tip of a P20 Pipetman. The mixture was then dropped into 10 μl of distilled water on a glow discharged glass slide that had been dipped in a solution of 0.6% poly(styrene-*co*-acrylonitrile) (Aldrich) in dichloroethane and then air dried. An additional 10 μl of bursting medium was added

to the slide, and then it was carried to a hood. For fixation, slides were initially sprayed with an aqueous mist of 4% paraformaldehyde (pH 8.5). After drying, the slides were flooded with 4% paraformaldehyde. Ten minutes later the paraformaldehyde solution was poured off, the slides were washed in distilled water, dipped in 0.1% Photoflo 200, and air dried. The following day, the slides were stained in one of two ways: For silver staining, aqueous 33% silver nitrate was placed on the SC spreads, covered with nylon screen (Tetko), and incubated in a humid chamber at 40°C for 10 min. After staining, the nylon screen was washed off with water, and the slide was allowed to air dry (Sherman *et al.* 1992). For uranyl acetate–lead citrate (UP) staining, slides were stained for 5 min in aqueous 2% uranyl acetate, washed in water, stained for 5 min in Reynold's lead citrate, washed in water, and allowed to air dry. Sets of well-spread SCs were located by phase-contrast light microscopy, picked up on grids, and examined and photographed at a magnification between 1.6K and 10K using an AEI 801 electron microscope.

Spreads of complete sets of zygotene and pachytene bivalents generally required from 2 to 6 electron micrographs for complete coverage. Completely analyzable bivalents in spreads were measured, and the location of ENs and LNs were determined by computer as described by Anderson *et al.* (2001). When zygotene bivalents were measured, only one of the two homologous AEs was measured through asynapsed areas, and this was referred to as the axial element pair length. ENs associated with either AE in a pair were counted as belonging to the pair. Two cases of completely synapsed bivalents were analyzed as zygotene bivalents because other bivalents in the set from which they came had not completed synapsis (Table 1). Twenty-one zygotene bivalents from 6 sets and 100 pachytene bivalents from 10 sets were analyzed.

Table 1. Summary of synapsis and EN data from 21 zygotene bivalents from KYS and *In7a*.

Bivalent ID number	Absolute length in μm	Synapsed length in μm (%)	No. ENs	No. ENs on SC	No. ENs per μm of SC	No. ENs per μm AE pairs
Zm00160-1	73.3	51.1 (70)	25	22	0.431	0.136
Zm00150-1	81.6	48.7 (60)	24	20	0.411	0.123
Zm00150-2	60.3	47.7 (79)	22	20	0.419	0.158
Zm00150-3	59.1	41.6 (70)	18	16	0.385	0.114
Zm00150-4	47.5	39.0 (82)	20	15	0.385	0.588
Zm00147-1	56.5	36.9 (65)	18	15	0.407	0.153
Zm00146-1	89.4	55.2 (62)	21	18	0.326	0.088
Zm00146-3	73.0	29.5 (40)	21	8	0.271	0.300
Zm00146-4	55.7	33.8 (61)	17	14	0.414	0.137
KYS total	566.4	383.4 (68)	186	147	0.383	0.213
ZmIn7a063-1	118.1	68.5 (58)	38	36	0.526	0.040
ZmIn7a063-2	82.6	55.2 (67)	36	34	0.616	0.073
ZmIn7a063-4	77.0	64.6 (84)	29	28	0.433	0.081
ZmIn7a063-5	76.5	47.5 (62)	19	15	0.316	0.138
ZmIn7a063-6	61.5	34.8 (57)	25	22	0.632	0.112
ZmIn7a063-7	60.0	21.7 (36)	13	9	0.415	0.104
ZmIn7a063-8	56.1	36.9 (66)	17	17	0.461	0.000
ZmIn7a063-9	52.9	52.9 (100)	22	22	0.416	0.000
ZmIn7a063-10	52.0	39.9 (77)	21	16	0.401	0.413
ZmIn7a061-1	84.2	61.3 (73)	47	47	0.768	0.000
ZmIn7a061-2	63.5	63.5 (100)	33	33	0.520	0.000
ZmIn7a061-3	52.4	34.0 (65)	26	25	0.735	0.054
In7a total	866.7	580.6 (67)	324	302	0.520	0.077
Total	1433.1	964.1 (67)	510	449	0.466	0.13

Results

During early zygotene, synapsis is typically initiated at or near the ends of maize chromosomes (Gillies 1975, Burnham 1980). Figure 1a shows a complete set of silver-stained early zygotene SCs in which sixteen of twenty pairs of chromosome ends are synapsed. Later in zygotene there are many examples of interstitial initiation of synapsis as well (Figure 1b). While not obvious in every SC spread (Figure 1a), chromosome ends are often clustered to one side in the bouquet orientation (Figure 1b; Bass *et al.* 2000).

After uranyl acetate–lead citrate staining of zygotene SCs, ENs can be seen in association with both unsynapsed axial elements and SCs (Figure 2). Because interpretable SC spreads at zygotene from KYS maize were rare, we decided to include observations of zygotene bivalents from a separate study involving a plant that was heterozygous for inversion *In7a* in KYS background. However, we found that the average frequency of ENs per unit length of SC is higher for the inversion heterozygote (0.52 EN/μm SC) than for the KYS plant (0.38 EN/μm SC) (Table 1). To test whether this difference is statistically significant, we compared the slopes of the regressions of EN numbers and SC segment lengths for each group (Albini & Jones 1987). The difference proved to be not significant (Table 2; $t = 0.929$; $df = 75$, $p > 0.5$; Zar 1984). As an alternative means of comparing the two data sets, we determined the average frequency of ENs per micrometer of SC for each SC segment using the ratio of the observed number of ENs on a segment to the segment's length in micrometers. By this method, both KYS and the inversion heterozygote averaged 0.421 ENs/μm of SC, and these averages were not significantly different ($t = -0.005$, $df = 77$, $p > 0.5$). Based on these results, the data from the KYS plant and the *In7a* inversion heterozygote were pooled for all further analyses. We did not use any bivalents that showed inversion loops.

The average (pooled) EN frequency per SC length during zygotene is 0.47 ENs/μm of SC compared with 0.13 ENs/μm of AE pair (see Materials and methods; Table 1, Figure 2). Therefore on average, there are about (0.47/0.13) 3.6 times more ENs per unit length of SC than per unit length of axial element pairs. Due to the high frequency of ENs on SCs compared with AE pairs and the fact that most zygotene bivalents that we examined were synapsed over a large fraction of their length (mean = 67% synapsis), more than 88% of the ENs that we observed were associated with SC (Table 1).

There is a strong positive correlation between SC segment length and the number of ENs on a segment, (Table 2, Figure 3). The regression has an r^2 of 0.81, indicating that approximately 80% of the variation in EN numbers on SC segments is predicted by the length of the SC segment.

We were interested in determining the placement of ENs along the length of maize zygotene bivalents. However, because kinetochores are not visible at zygotene, we were not able to distinguish long from short arms, so we were unable to identify individual bivalents (based on arm ratios). An alternative approach to describe the location of ENs on bivalents is to estimate the frequency of ENs on SC segments from distal to medial. (The term proximal (meaning closer to the centromere) cannot be used here because centromeres (kinetochores) are not visible during zygotene.) For this, each zygotene bivalent was measured and divided into twenty equal intervals representing 5% of the total bivalent length. Each

Table 2. Summary of regression equations for EN number and SC segment length.

	Slope			Intercept			Reg MS (df)[a]	Res MS (df)[a]	r^2
	Coefficient	SD	*p* value	Coefficient	SD	*p* value			
KYS	0.36	0.04	<0.001	0.42	0.57	0.46	344.7 (1)	4.12 (32)	0.72
In7a	0.56	0.04	<0.001	−0.40	0.69	0.57	2814.4 (1)	11.8 (43)	0.85
Pooled: KYS & *In7a*	0.52	0.03	<0.001	−0.50	0.50	0.31	3144.1 (1)	9.8 (77)	0.81

[a]Reg MS, regression mean square; Res MS, residual mean square; df, degrees of freedom.

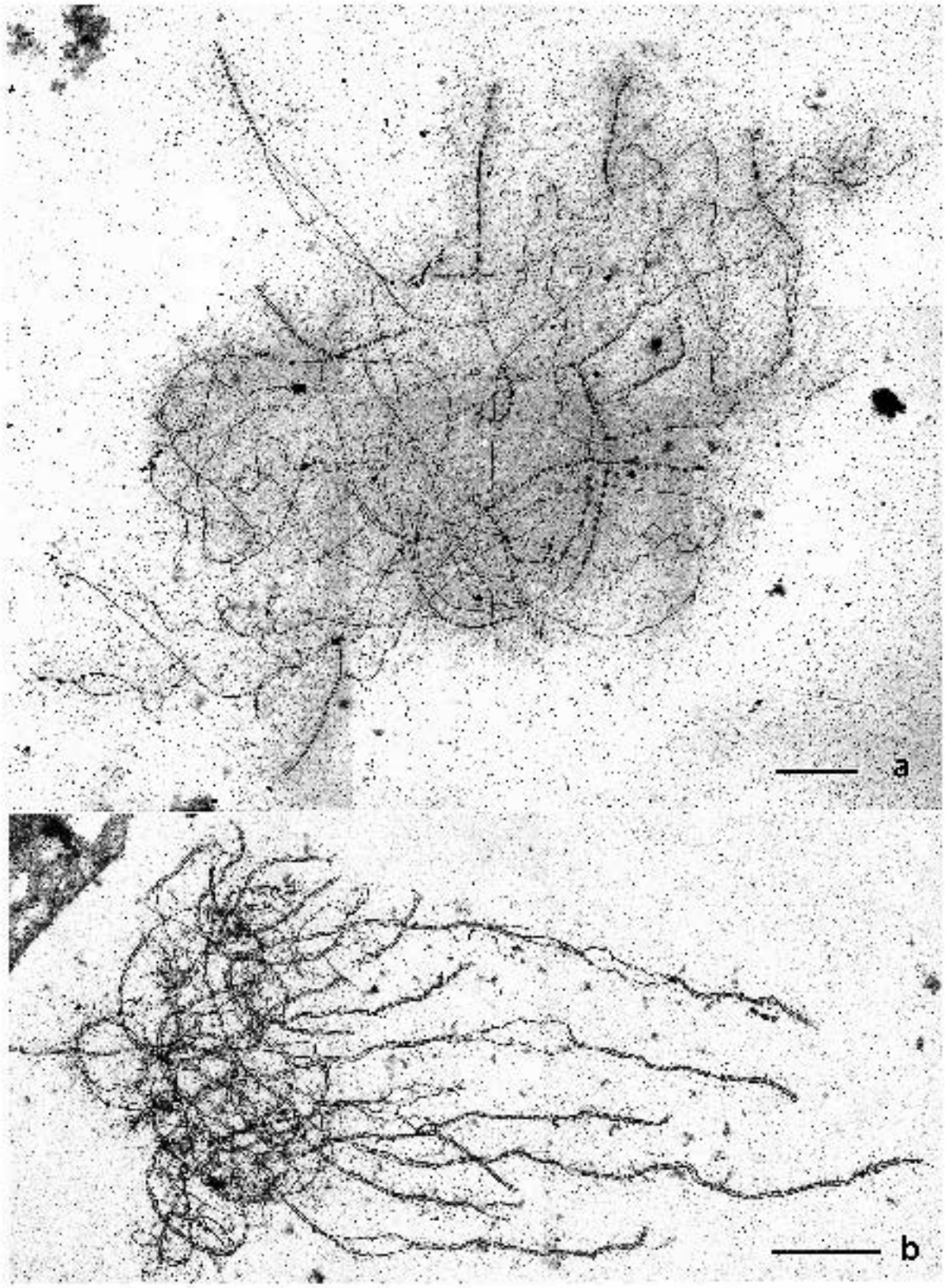

Figure 1. Complete sets of silver-stained zygotene SCs. (**a**) Early zygotene with sixteen of twenty pairs of chromosome ends synapsed. (**b**) Later zygotene with most of the ends to one side in the bouquet orientation. While the ends of bivalents are generally synapsed, there are many sites of interstitial synapsis as well. Bars = 5 μm.

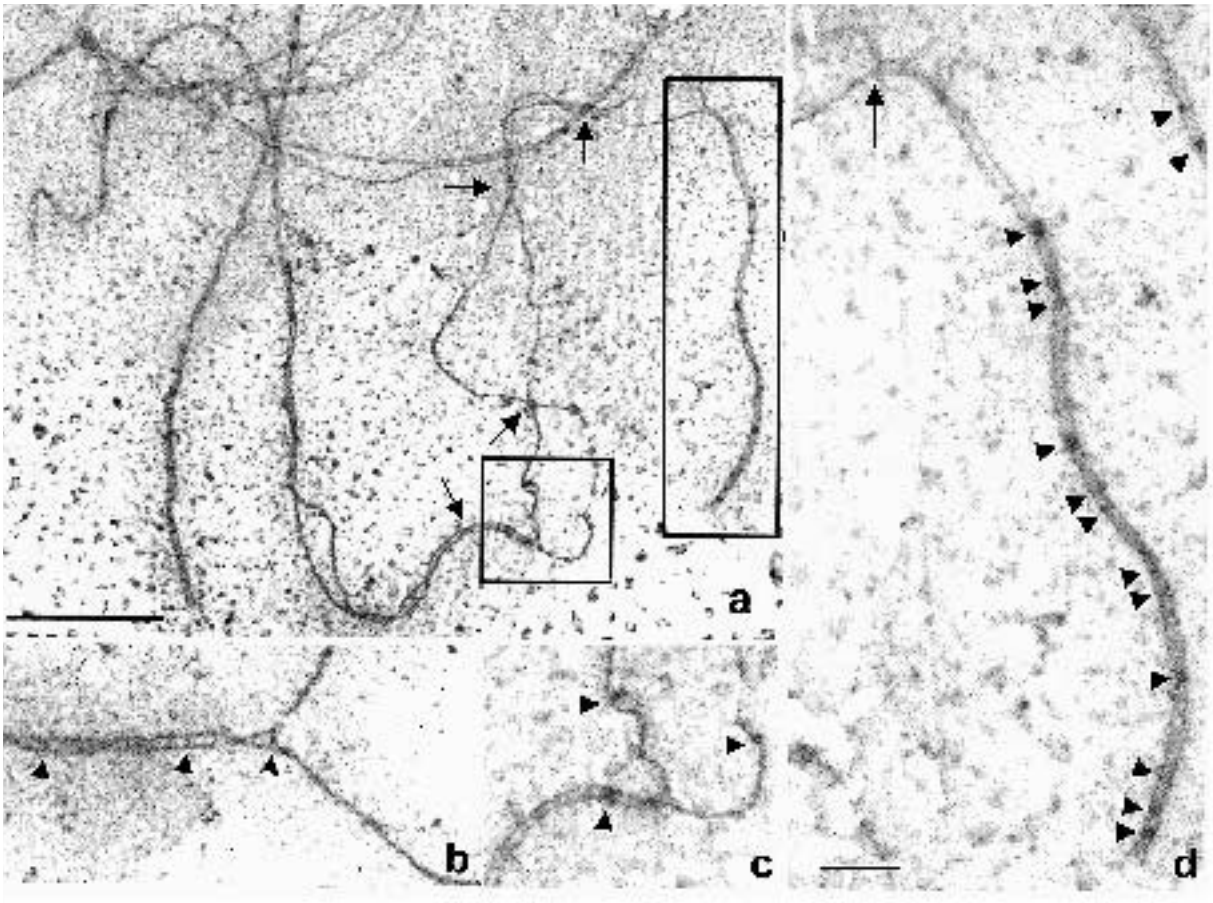

Figure 2. UP-stained SCs at zygotene. (**a**) A complete bivalent with synapsed ends and interstitial sites of synaptic initiation (arrows). Bar = 5 μm. (**b**) Segment of an SC with ENs (arrowheads). One EN is at a synaptic fork (right). (**c**) Magnified view of the small boxed area in (**a**) showing a synaptic fork without an EN. ENs on SC and on axial elements are indicated by arrowheads. (**d**) Magnified view of the large boxed area in (**a**) showing ENs on SC (arrowheads). ENs vary considerably in size and shape. Note the synaptic fork without an EN (arrow). In (**b**–**d**) the bar = 1 μm.

interval was numbered from 10 (most distal) to 1 (most medial) (Figure 4). The length of SC in each interval was calculated (if synapsis had occurred in the interval), and the number of ENs on SC in each interval was counted. Data from segments with the same interval number (and that have the same relative position to either side of the middle of the bivalent) were combined to form ten categories, each category representing up to (5% + 5%) 10% of a bivalent's length. The total number of ENs observed in a category was divided by the total length of SC in the category to give the frequency of ENs per micrometer of SC for the 21 zygotene bivalents analyzed (Table 3). Typically, zygotene bivalents are more often synapsed at the ends than in the middle, so more length of SC was analyzed in more distal categories than in more proximal categories. The frequency of ENs varied along the length of SCs, with the highest frequency (0.69 EN/μm SC) occurring in the most distal category (10) and the lowest frequency (0.27 EN/μm SC) occurring in a more medial category (4) (Table 3, Figures 4 & 5). The likely reason that the most medial category (1) does not have the lowest frequency of ENs is that most maize chromosomes are not metacentric (Anderson & Stack 2001), so the average position of the centromere (kinetochore) over

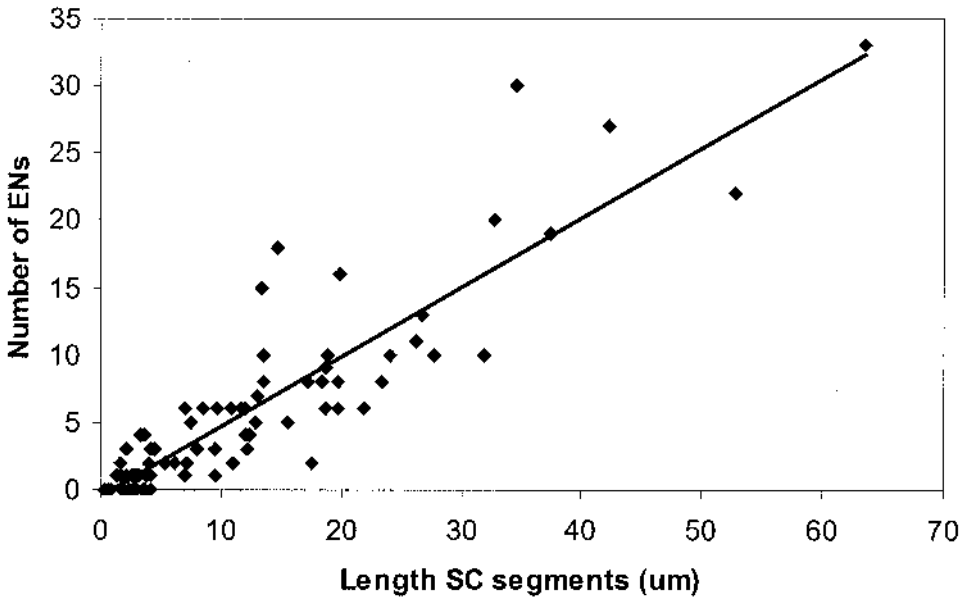

Figure 3. Plot of the number of ENs on a segment of SC versus the length of the SC segment in micrometers for KYS and *In7a* zygotene bivalents. The regression line is described by the formula $y = 0.52x - 0.50$ ($r^2 = 0.81$).

all ten maize bivalents falls in category 4 (Figure 4), where the lowest concentration of ENs is observed (0.27 ENs/μm of SC; Figure 5).

Although the distal segments had a high frequency of ENs, the highest frequency of ENs per unit length of SC occurs at synaptic forks, defined as sites of transition between synapsis (formed SC) and asynapsis (axial elements) (Figure 2). This was determined by examining 110 synaptic forks and observing the presence of an EN 28 times in the 0.1 μm of SC at the forks. This yields a frequency of (28 ENs/11.0 μm of SC) 2.55 ENs/μm of SC, a frequency that is (2.55/0.69) 3.7-fold higher than in distal category 10 and (2.55/0.27) 9.4-fold higher than in the more medial category 4.

To determine if ENs are continually added to SC segments throughout zygotene, we looked for a

Table 3. Comparison of the frequency of ENs to LNs for ten categories representing combined 5% + 5% SC segment length intervals from the middle (interval 1) to the ends (interval 10) of twenty-one zygotene bivalents from KYS and In7a and 100 pachytene bivalents from KYS.

Nodules	1	2	3	4	5	6	7	8	9	10	Total
Early (n = 21 bivalents)											
Number of ENs	22	22	26	21	42	44	48	61	73	93	452
Absolute length of synapsed SC (μm)	61.5	61.9	64.6	77.6	91.8	104.3	110.2	125.3	130.7	135.6	963.6
Number of ENs per μm SC	0.36	0.36	0.40	0.27	0.46	0.42	0.44	0.49	0.56	0.69	0.47
Late (n = 100 bivalents)											
Number of LNs	11	7	7	7	19	12	13	33	48	61	218
Absolute length of synapsed SC (μm)	338	338	338	338	338	338	338	338	338	338	3380
Number of LNs per μm SC	0.03	0.02	0.02	0.02	0.06	0.04	0.04	0.10	0.14	0.18	0.06
Ratio of EN to LN frequency	12	18	20	14	8	11	11	5	4	4	8

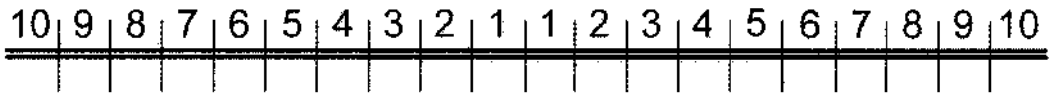

Figure 4. Diagram of a zygotene bivalent (double horizontal line) divided into 5% intervals that are numbered from 10 (most distal) to 1 (most medial). Since kinetochores are not visible and individual bivalents could not be identified, like numbered segments (together representing 10% of the length of the bivalent) were combined to determine frequency of ENs at comparable distances from the ends of bivalents.

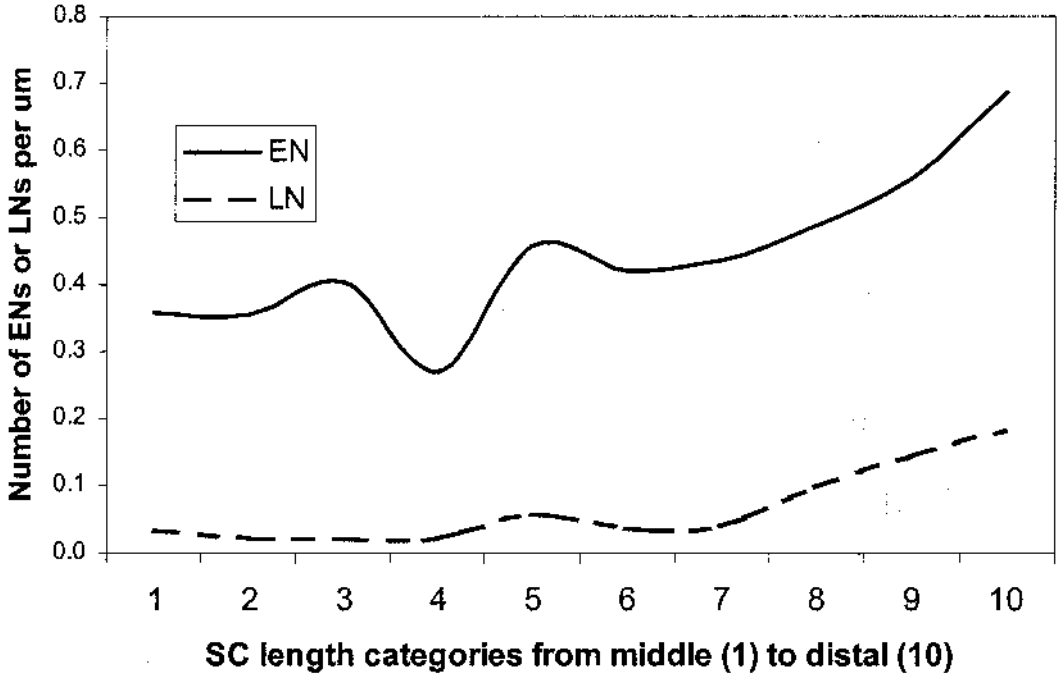

Figure 5. Graph of EN (solid line) and LN (dashed line) frequencies per micrometer of SC from medial (left) to distal (right) during zygotene and pachytene, respectively. Each category is comprised of two 5% lengths with the same number and relative position on a bivalent (Figure 4). Both ENs and LNs occur more frequently in distal (toward interval 10) than in medial categories (toward interval 1). Note the dip in EN frequency in category 4 on the X axis. This corresponds to the average location of centromeres on maize chromosomes.

change in the frequency of ENs per unit length of SC from middle to late zygotene. The rationale is that if ENs continue to associate with formed SC, the frequency of ENs per unit length of SC should increase from early to late zygotene. The stage of zygotene was estimated by the fraction of a bivalent's length that was synapsed: the higher the percentage of synapsis, the later in zygotene the bivalent was assumed to be. For a valid analysis, comparable segments of SCs must be analyzed, so we looked at the frequency of ENs per unit length of SC in the two distal 20% segments of each bivalent (40% of total bivalent length). These segments were selected for analysis because they synapse first so they would have the most time to collect additional ENs as zygotene progresses. The data and regression are plotted in Figure 6. The slope of the regression is not significantly different from zero ($y = 0.001x + 0.49$, $r^2 = 0.003$, $p = 0.797$), indicating that the frequency of ENs per unit length of distal segments of SC is not related to stage of zygotene

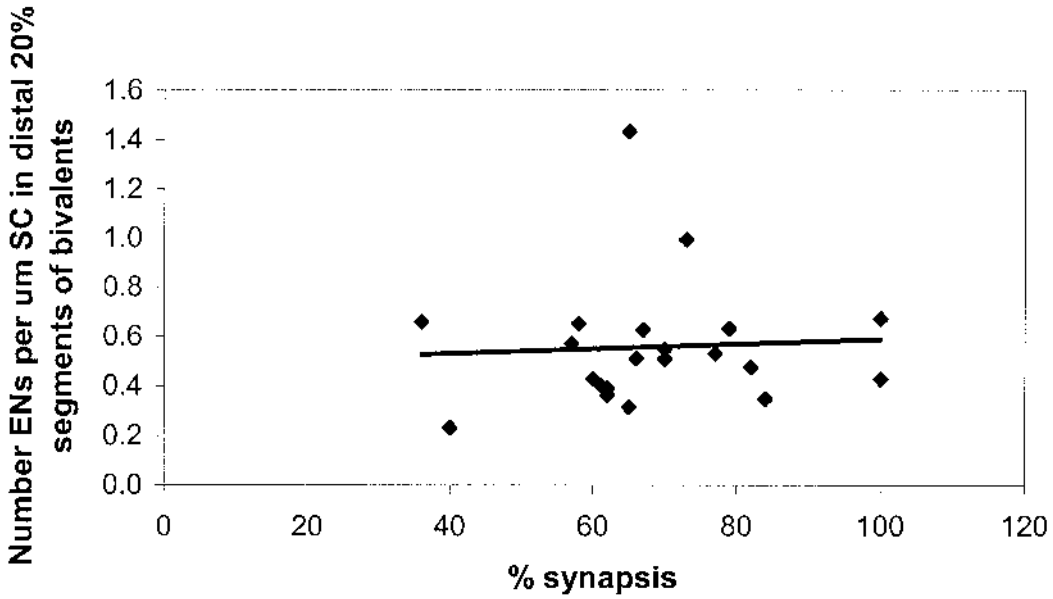

Figure 6. The frequency of ENs per micrometer of SC in the two distal 20% segments of a bivalent compared to the fraction (per cent) synapsis of the bivalent. The regression line is described by the formula $y = 0.001x + 0.492$, $r^2 = 0.003$.

as judged by the total amount of synapsis. We interpret this to mean that additional ENs are not added to formed SC.

Distances between adjacent ENs on SCs were measured to determine if the presence of an EN influences the position of adjacent ENs, i.e. do maize ENs show interference? Because the frequency of ENs is low on axial elements, we measured distances only between adjacent ENs on the same synapsed segment of chromosome. Distances between adjacent ENs on asynapsed segments, distances between adjacent ENs on synapsed segments that were separated by asynapsis, and distances between adjacent ENs on synapsed and asynapsed segments were not included in the analysis. The mean distance between adjacent ENs is 1.77 μm (n = 393; Table 4, Figure 7). However, the distribution is skewed to the left, and the median distance between ENs is much lower (1.20 μm; Table 4). The observed distribution of separation distances was compared with two types of random distributions (normal and gamma) predicted from parameters derived from the data (Figure 7; Anderson *et al.* 2001). While a visual inspection of the curves suggests that the gamma distribution is a much closer fit to the observed distribution than the normal distribution, statistical analysis indicates that the observed distribution differs significantly from both predicted distributions (Anderson-Darling normality test ($p>0.05$) and Kolmogorov–Smirnov one sample goodness of fit test ($p>0.05$)). However, there are two fairly distinct classes of data that were used together to generate the curves. One class, representing the majority of the data, was obtained from distal segments of SC where the frequency of ENs is generally higher. The other class was obtained from proximal segments near centromeres where synapsis occurs last and ENs are less common (Table 3, Figure 5). To see if the distribution changed when only distal segments were considered, we reanalyzed the data using the distal 10% segments (20% of the total length) of each bivalent (Table 4). When this more limited data set (n=125) was plotted and compared with normal and gamma distributions, the observed distribution still differed significantly from the normal distribution, but the observed distribution was not significantly different from a gamma distribution ($p< 0.05$; Figure 8). Even so, examples of separation distances $\leq$0.2 μm are conspicuously missing in both data sets. This may be due partly to the fact that the distances between ENs are measured from center

Table 4. Summary statistics for distances between adjacent ENs on the same SC segment from zygotene bivalents.

	Distances between adjacent ENs (μm)	
	Entire bivalent length	Two distal 10% lengths of each SC
No. observations	393	125
Mean	1.77	1.20
Standard Deviation	1.79	0.97
Median	1.20	0.83
Alpha	0.98	1.51
Beta	1.81	0.79

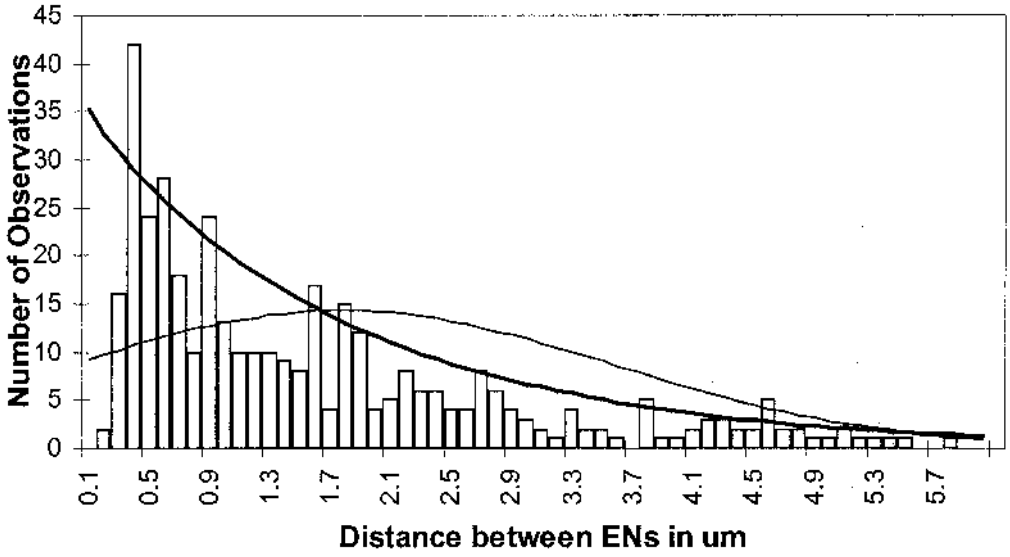

Figure 7. Histogram of distances between adjacent ENs on continuous segments of SC (n = 393). There were fourteen observations greater than six micrometers that were not included in the graph. The observed distribution of separation distances (columns) was compared to normal (thin line) and gamma distributions (thick line) predicted from parameters derived from the data (see Anderson *et al.* 2001). By a visual inspection, the gamma distribution fits the observed distribution much better than the normal distribution, except for distances $\leq 0.2\,\mu$m of which there are only two observations. The observed distribution differs statistically from both the normal and gamma distributions at the 5% level.

to center, so two ENs (each approximately 0.1 μm in diameter) that are separated by $\leq$0.1 μm would touch or overlap and may have been counted as one EN. Indeed, if the 0.2 μm interval is eliminated from the complete data set (Figure 7), it also fits a gamma distribution (not illustrated). This indicates that, possibly aside from separation distances of $\leq$0.2 μm, ENs do not show interference.

To see if there is a relationship between the distribution of ENs at zygotene and the distribution of LNs (crossovers) at pachytene, we examined the distribution of LNs on ten complete sets of late pachytene SCs (100 pachytene bivalents) (Figure 9). The average number of LNs per set was 21.9, a number similar to previous reports of the average numbers of chiasmata per chromosome set at diakinesis in maize (18 – Beadle 1933; 27– Darlington 1934). Generally the frequency of LNs per unit length of SC is much (on average 8-fold) lower on late pachytene SCs compared with ENs on zygotene SCs (Table 3). When frequencies of LNs on SCs in (5% + 5%) 10% length categories from distal to medial are calculated (just as was

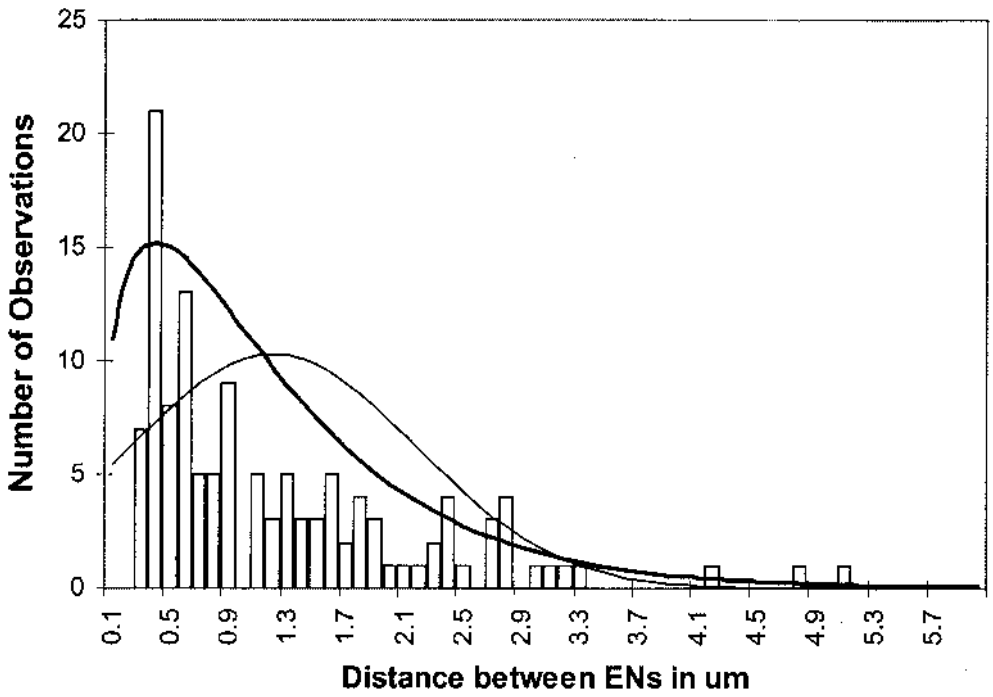

Figure 8. Histogram of distances between adjacent ENs on SC in the distal 10% segments of zygotene bivalents (n = 125). The observed distribution of separation distances (columns) was compared to normal (thin line) and gamma distributions (thick line) predicted from parameters derived from the data. Using this subset of the data, the observed distribution is not significantly different from the gamma distribution at the 5% confidence level.

done for ENs in zygotene), higher frequencies of LNs occur distally and lower frequencies occur medially, similar to the pattern observed for ENs (Table 3, Figure 5). However, the ratio of EN to LN frequencies is not constant along the length of bivalents. ENs are only 4-fold more common than LNs in distal segments, while ENs are up to 20-fold more common than LNs in medial segments (Table 3).

To determine if LNs show interference, we analyzed twenty cases among 100 pachytene bivalents where two LNs were observed on long arms (identifiable because kinetochores are visible in pachytene; Figure 9). If LNs are distributed at random throughout an arm, one would expect an average separation distance of 1/3 (33%) of the arm (Carpenter 1988), although two factors should reduce this distance. First, LNs are concentrated distally so this should result in shorter distances between LNs, and second, any LNs in the short arm would tend to push LNs in the long arm closer together if interference extends through the centromere, as observed by Colombo & Jones (1997) in grasshoppers. However, the average separation distance between pairs of nodules in long arms was observed to be 47% (SD 21%, range 7–83%) of the arm length, so it appears that, unlike ENs, LNs show interference.

Discussion

During zygotene in maize, chromosomes are often observed in the bouquet orientation, and synapsis is initiated distally. These are common patterns that have been reported earlier in maize and other plants (e.g. Gillies 1975, Holm 1977, Burnham 1980, Stack & Anderson 1986a, Bass *et al.* 2000). Active movement of telomeres into a limited area of the nuclear envelope (the bouquet orientation) may be a means of roughly positioning chromosomes in preparation for presynaptic alignment (Maguire 1983, Loidl 1988) and initiation of homologous synapsis near chromosome ends (see

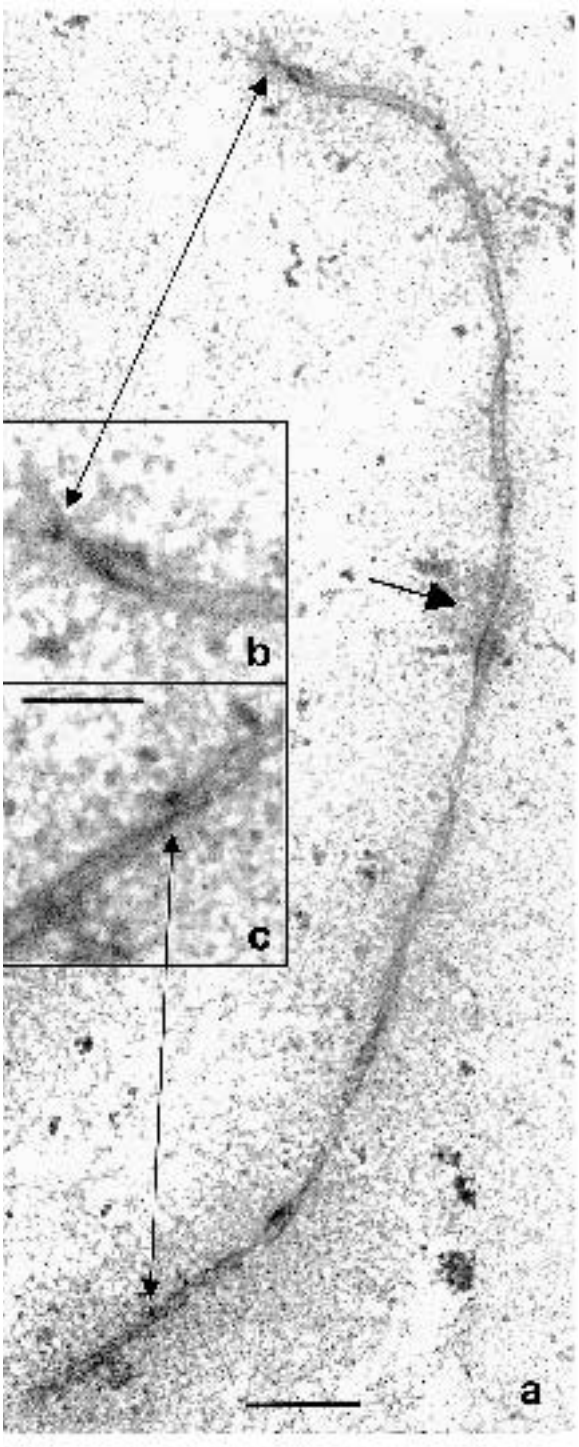

Figure 9. (**a**) UP-stained late pachytene SC. The kinetochore is indicated by an arrow. Two LNs are visible. One is located at the upper end of the SC, and another is located near a twist of the lateral elements at the lower end of the SC (arrows). Bar = 2 μm. (**b, c**) Both LNs are illustrated at higher magnifications in the insets. Bar = 1 μm.

Zickler & Kleckner 1998 for a review). The importance of aligning chromosome ends for subsequent synapsis and crossing over is suggested by reports in wheat of crossover suppression between homologous chromosome arms that differ in length due to heterozygosity for terminal deficiencies (Curtis *et al.* 1991, Lukasjewski 1997).

We also find that the general pattern of ENs on maize SCs during zygotene is similar to those we recently reported for other plant species (*Lycopersicon esculentum, Cyphomandra betacea, Allium cepa, Tradescantia edwardsiana, Lilium longiflorum*, and *Psilotum nudum*) (Anderson *et al.* 2001; also see Albini & Jones 1987 for observations of *A. cepa* and *A. fistulosum*). For example:

(1) The frequency of ENs per unit length of homologous axial element pairs is lower than the frequency of ENs per unit length of SC during zygotene (see (3) below). For maize there are 3.6-fold fewer ENs per unit length of AE pairs. This value is within the range reported for other plants (Anderson *et al.* 2001). While it is possible that this difference is due in part to contraction of axial elements as they are converted to lateral elements during synapsis, we have no evidence that axial elements change in length, and a 3.6-fold contraction of axial elements to explain all of the difference in EN frequency on axial element pairs and zygotene SC seems improbable. Furthermore, if lateral elements shorten during zygotene, this should result in an increasing frequency of ENs per unit length of SC as zygotene progresses, and this does not appear to be the case (see (2) below). Alternatively, the difference could be due to preferential loss of ENs from axial elements during spreading but again we have no independent evidence that this occurs.

(2) There is a good correlation between the length of an SC segment in zygotene and the number of ENs on the SC segment. Albini & Jones (1987) made similar observations for *Allium cepa* and *A. fistulosum*.

(3) The frequency of ENs per unit length of zygotene SC does not increase or decrease as zygotene progresses. This can be explained if ENs do not continue to associate with SC

after it is formed or if ENs are added and lost from formed SC at the same rate throughout zygotene. We think the former explanation is more likely since it requires no complex controls.

(4) The highest frequency of ENs occurs at synaptic forks. This is most easily explained if ENs assemble at synaptic forks and, during assembly, synapsis is delayed. If synapsis were not delayed during assembly of ENs, the frequency of ENs at synaptic forks would be the same as on formed SC. The relationship between ENs associated with AEs prior to synapsis and ENs associated with SC is not clear. However, the fact that at least some of both types of ENs can be labeled with an antibody to Rad51 protein suggests that they are comparable structures (Anderson *et al.* 1997). Possibly all ENs assemble on axial elements but (to explain the difference in numbers see (1) above) some ENs on axial elements are lost in the spreading procedure while ENs on SC are retained. However, it is not clear why some ENs would be lost from maize AEs while others would be retained, and lily spreads do not appear to lose ENs from AEs (Anderson *et al*. 2001). In any case, if ENs do attach exclusively to AEs, synaptic progress would still have to be delayed when synaptic forks encounter ENs on axial elements to explain the high frequency of ENs at forks.

It should be mentioned that, in maize microsporocytes, Franklin *et al.* (1999) observed up to $\sim$700 fluorescent foci labeled with antibodies to Rad51p, which were associated with zygotene bivalents. While it is tempting to consider these foci to be ENs, there are discrepancies between Rad51 foci and our observations of ENs by electron microscopy. For example, the fluorescent foci are more numerous than the ENs we observe in SC spreads. At late zygotene when synapsis is essentially complete, we expect a maximum of about (350 μm of SC $\times$ 0.47 EN/ μm SC) 165 ENs associated with SC per nucleus in comparison with a maximum of $\sim$700 Rad51 foci observed by Franklin *et al.* (1999) at middle zygotene. Also the fluorescent foci are concentrated in unsynapsed parts of chromosomes, whereas the ENs we observe are concentrated on SC. Finally, many of the fluorescent foci are paired structures, while the ENs we observe are seldom paired. A number of explanations can be offered for these discrepancies but, considering the observation by Anderson *et al.* (1997) that not all ENs associated with lily zygotene bivalents are labeled with an antibody to Rad51p and the fact that there are two closely related *Rad51* genes in maize (Franklin et al. 1999), it is not clear that all Rad51 fluorescent foci correspond to ENs and *vice versa*

(5) ENs show little, if any, interference with each other. The evidence for this is that separation distances between adjacent ENs on SC segments come close to a gamma-type of random distribution. While this pattern could be generated by a random distribution of ENs on AEs, the low number of ENs on AEs and the high frequency of ENs at synaptic forks suggest that most ENs assemble randomly over time at forks (Anderson *et al.* 2001). In this situation, a gamma distribution can arise when SC formation progresses for variable distances before another EN is assembled at a fork.

The observed distribution of separation distances fits a gamma distribution best if the shortest measured interval separating ENs ($\leq$0.2 μm), which has too few observations, is ignored. This can be explained if maize ENs interfere with each other only within this short interval or if overlap of ENs results in undercounting of EN pairs separated by $\leq$0.2 μm (see Results). Other plant species also show this deficiency of close EN pairs (Anderson *et al.* 2001). On the other hand, even though EN separation distances generally fit a gamma type of random distribution, ENs are not randomly distributed on the large scale of whole maize bivalents because ENs are more concentrated distally (Table 3, Figure 5; also see Albini & Jones 1987 for similar observations on *A. cepa*).

(6) By pachytene, ENs are lost from SCs, and their place is taken by much less numerous LNs. LNs occur at sites of crossing over

and show interference comparable to that reported for LNs in tomato (Sherman & Stack 1995), crossovers (e.g. Muller 1916), and chiasmata (e.g. Mather 1933, Jones 1984). LNs remain visible through pachytene and into early diplotene when chiasmata take their place (Stack & Anderson 1986a, Albini & Jones 1987, Anderson & Stack 1988, Stack & Roelofs 1996).

In this study of maize RNs we found that there is a gradient in the frequency of ENs per unit length of SC from the highest frequency (0.69 ENs/μm) in the most distal category of segments that tend to synapse early in zygotene to the more medial categories of segments (0.27–0.40 ENs/μm) that tend to synapse later in zygotene (Table 3, Figures 4 & 5). Probably, the reason that the medial category 1 does not have the lowest frequency of ENs is that most maize chromosomes are not metacentric (Anderson & Stack 2001) so the average position of the centromere (kinetochore) falls in category 4 (Figures 4 & 5) where the lowest concentration of ENs is observed (0.27 ENs/μm of SC). Considering that this category is composed of two 5% segments on either side of the middle of bivalents, the real concentration of ENs around centromeres is probably even lower. LNs show a similar gradient but the relative frequency of LNs through the more medial categories is even more suppressed than the relative frequency of ENs (Figure 5).

What could account for the gradients of EN and LN frequency along bivalents? One possibility is that ENs might continually associate with formed SC, so distal SC segments that are synapsed for longer would have more time to collect ENs than proximal segments that synapse later. However, this is unlikely because we found that ENs do not increase in frequency per unit length in distal SC segments as zygotene progresses, i.e., ENs are not added to already formed SCs. Another option is that EN components may be in limited supply, and most of the components are exhausted by associating with the first formed distal segments of SC. While we cannot eliminate this possibility, we think the more likely explanation is related to the location of genes on maize chromosomes. If maize is like other cereals with large genomes (wheat – Gill *et al.* 1993, barley – Künzel *et al.* 2000), most maize genes are concentrated distally. Since there is a tendency for DNA double-strand breaks and crossing over to occur in genes (Thuriaux 1977, Dooner 1986, Thomas & Rothstein 1989, Okagaki & Weil 1997; probably due to the open configuration of chromatin in active genes – Wu & Lichten 1994, Virgin *et al.* 1995) and ENs and LNs appear to be involved in these events, one would expect ENs and LNs to be concentrated distally as well.

While the function of the numerous ENs observed during zygotene is unclear, there is substantial evidence that a subset of ENs become LNs (von Wettstein *et al.* 1984, Stack & Anderson 1986b, Sherman *et al.* 1992, Plug *et al.* 1998, Zickler & Kleckner 1999, Agarwal & Roeder 2000, Ashley & Plug 1998, Novak *et al.* 2001). If so, the efficiency of this transformation in maize is higher in distal SC segments than in more medial segments (Figure 5, Table 3). For example, in distal segments, four ENs were observed for every LN while, in more medial segments, up to 20 ENs were observed for every LN. To explain this, we note that distal segments of chromosomes usually synapse first, and we propose that distal ENs are assembled and begin the process leading to crossing over earlier than ENs that are assembled later on more proximal segments of SC. When an EN is successful in achieving a crossover and becomes an LN, interference emanating from the crossover site suppresses nearby recombination activity in ENs. The loss of ENs (that do not show interference) near successful crossover sites (LNs that show interference) is a physical manifestation of crossover interference (Rasmussen & Holm 1978, Stack & Anderson 1986b). The result would be more crossing over distally than proximally, just as is observed in maize (Rhoades 1955, Dempsey 1994). Thus the prediction is that segments of chromosomes that synapse earlier will tend to have more crossing over than segments of chromosomes that synapse later (Curtis *et al* 1991). We suggest that this pattern is the norm (e.g., Anderson *et al.* 1999), but it is not universal (see Jones 1984, 1987 for discussions of the relationship between synapsis and crossing over/chiasmata).

In cases where the pattern of synapsis does not seem to correlate with the pattern of crossing over, there must be additional factors in operation, e.g.,

certain *Allium* (Levan 1933, 1935, Jones 1984, Albini & Jones 1987, 1988, Stack & Roelofs 1996) and newt species (Callan & Perry 1977) that show proximal localization of crossing over in spite of distal initiation of synapsis. While other explanations are possible in the context of the scheme presented above, such a pattern of synapsis and crossing over could be explained if ENs formed early during synapsis (distally) lack a critical component (protein) for crossing over that is only available late in zygotene when proximal synapsis occurs and proximal ENs form. Since new ENs do not appear to be added to formed SC, distal SC would have faulty ENs that cannot achieve crossovers, while proximal ENs are functional and reliably form crossovers without interference from distal crossover sites.

The preceding scheme assumes that, typically, all ENs are the same initially, but ENs have different fates depending on timing and location of successful crossover events. It is unclear whether ENs that do not participate in crossing over are capable of mediating other types of recombination events, such as gene conversion, or how the pattern of ENs we observe may be related to results from yeast suggesting separate pathways for crossing over and gene conversion (Allers & Lichten 2001).

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