# THE RELATIONSHIP BETWEEN SYNAPTINEMAL COMPLEXES, RECOMBINATION NODULES AND CROSSING OVER IN *NEUROSPORA CRASSA* BIVALENTS AND TRANSLOCATION QUADRIVALENTS

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ABSTRACT

Reconstruction of serially sectioned zygotene and pachytene nuclei has allowed the estimation of both the number and position of central component recombination nodules in the synaptinemal complexes of two chromosomally different strains of *Neurospora crassa*. In both strains the number of nodules is that expected if each nodule represents one crossover event (50 map units). The distribution of nodules within the arms of bivalents shows evidence of centromeric repulsion and telomeric localization. Nodules appear quite early in the zygotene before pairing of chromosomes is complete. Evidence was found of size differences in nodules, and multiple nodules were occasionally seen. Chromosome lengths and nuclear sizes increased from early zygotene to late pachytene. The three quadrivalents present in the *alcoy* translocation heterozygotes were readily distinguishable in reconstructions, and their cytological dimensions were in agreement with predictions from linkage map distances.

THE importance of genetic crossing over for both meiotic disjunction and reassortment of linked genes is indisputable. This has led numerous workers to investigate the mechanisms of recombination and its control (reviews, CATCHESIDE 1977; BAKER *et al.* 1976), usually by genetical or cytological analysis of the crossover products. Meiotic recombination is generally believed to occur at pachytene (HENDERSON 1970), and the discovery of the regular appearance of the synaptinemal complex at this stage (MOSES 1968) led to the suggestion that the synaptinemal complex was the medium for genetic crossing over. The evidence for this has become stronger (WESTERGAARD and VON WETTSTEIN 1972; GILLIES 1975), culminating in the description of *recombination nodules* associated with the synaptinemal complex in *Drosophila melanogaster* female meiosis (CARPENTER 1975). CARPENTER found that the recombination nodules were transient structures present only at pachytene, and she suggested that they were involved in recombination events. The number and position of recombination nodules correspond with expected crossover events, based on linkage map distances. In addition, they occurred only in euchromatin and exhibited a positive chiasma interference effect when two were present in one chromosome arm.

CARPENTER noted the similarity of the Drosophila nodules to the central component nodes that had been described in the synaptinemal complex of fungi (GILLIES 1972; ZICKLER 1973; RADU, STEINLAUF and KOLTIN 1974; SCHRANTZ 1970), and she suggested that these nodes might also be recombination specific. Nodes have subsequently been described in yeast (BYERS and GOETSCH 1975), where again they correlate in number and position with exchange events, Chlamydomonas (STORMS and HASTINGS 1977), Ascaris (BOGDANOV 1977) and Schizophyllum (CARMI *et al.* 1978), while nodules have been reported in the mouse (MOSES, RUSSELL and CACHEIRO 1977), rat (MOENS 1978), and in Bombyx and human males (RASMUSSEN, personal communication). ZICKLER (1977) has recently mapped the occurrence of central component nodules* in *Sordaria macrospora*. She found that they occur in all seven bivalents and correspond in number to the observed chiasma frequency.

In the original report on central component nodules in *Neurospora crassa* (GILLIES 1972), nodules were not found in all bivalents. It was suggested that this could be due to technical reasons. The results reported here concern further work on normal and rearranged Neurospora chromosomes in which the number and position of nodules have been closely studied.

## MATERIALS AND METHODS

Crosses of *Neurospora crassa* strains were carried out according to the method of BARRY (1966) on WESTERGAARD and MITCHELL's (1947) crossing medium fortified with 300 mg of lysine per liter. Two chromosomal constitutions were investigated. For the standard chromosome configuration, *asco* strain 37402a (FGSC No. 405) was crossed with wild type EMERSON 5256A (FGSC No. 626 or 424). The multiple translocation tester stock *alcoy a* (FGSC No. 998) was also crossed with EMERSON 5256A. *alcoy* (PERKINS *et al.* 1969) is a stock with three translocations involving six of the seven chromosomes: *T(I;II)*, *T(IV;V)*, *T(III;VI)*. Breakpoints defined by markers and the expected cross configurations expected in heterozygotes are diagrammed in PERKINS *et al.* (1969).

Perithecia were fixed for two to six hrs in 4 to 6.4% glutaraldehyde in phosphate buffer, pH 7.0, followed by 1.5 to two hrs in 2% osmium tetroxide in buffer. After washing in water, they were stained in 5% uranylacetate for six, eight, or 16 hr, washed again and dehydrated through alcohol series and propylene oxide. After the perithecia were infiltrated with SPURR's (1969) low-viscosity resin, individual asci were dissected out and embedded on glass slides by the method of WESTERGAARD and VON WETTSTEIN (1970). Serial sections of about 1000 Å thickness were cut with a diamond knife, picked up on formvar-coated single hole grids (GILLIES 1972) and post-stained for ten min with lead citrate (REYNOLDS 1963).

The ascal nuclei in the serial sections were photographed at constant magnification (usually 7000×) with a Philips EM200 or EM 201C instrument. Using photomicrographs (final magnification usually 20,000×), reconstructions of synaptinemal complex components were carried out as described previously (GILLIES 1972), with the positions of nodules being noted. Projected lengths and distances were corrected for section thickness by the Pythagorean method previously used (GILLIES 1972). For unpaired regions of bivalents and quadrivalents, the mean length of the two lateral components was used.

* Hereafter "nodules" will be used for both "nodes" and "nodules". "Central component" will be prefixed to distinguish ascomycete nodules, which lie inside the synaptinemal complex central region, from those described by CARPENTER (1975), which are usually alongside the synaptinemal complex.

## RESULTS

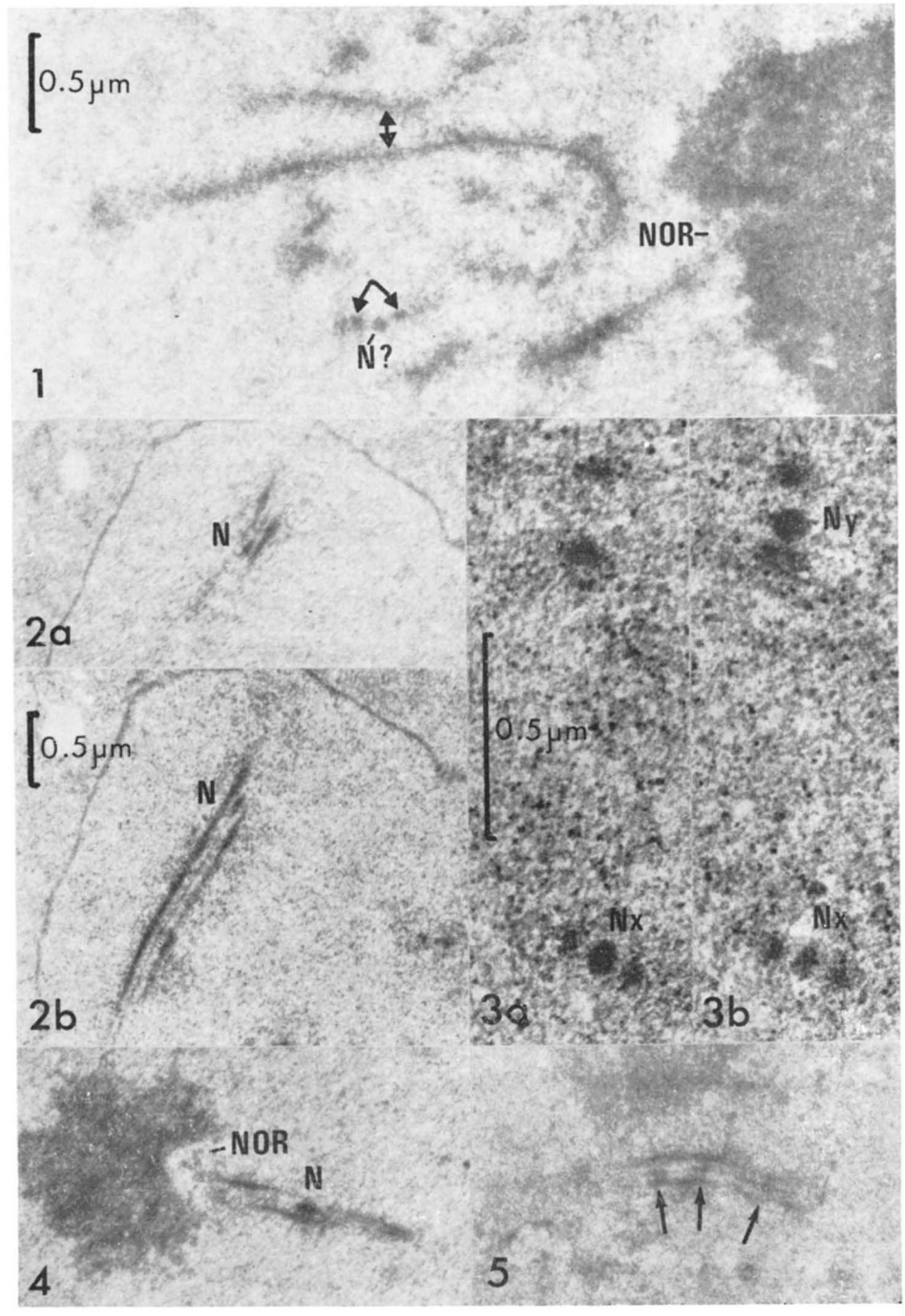
Sixteen *asco a* × Emerson *A* nuclei and nine *alcoy a* × Emerson *A* nuclei were serially sectioned, and of these, eight *asco* and seven *alcoy* were reconstructed completely. The remaining ten nuclei had sections missing and hence could only be partially reconstructed. The stages present ranged from early zygotene to late pachytene, based on size and shape of asci (BARRY 1969) and degree of synaptinemal complex formation. In addition one early diplotene *asco* nucleus and one leptotene *alcoy* nucleus were serially sectioned, but not successfully reconstructed.

The leptotene nucleus had a diameter of 4.2 $\mu$m and a volume of 38.8 $\mu m^3$. In eight zygotene-early pachytene nuclei of both genotypes the mean nuclear diameter was 4.74 $\mu$m, giving a nuclear volume of 55.83 $\mu m^3$. The nucleolar volume was 5.73 $\mu m^3$. In 17 pachytene nuclei the average nuclear diameter was 5.34 $\mu$m, and the volume was 79.8 $\mu m^3$. The nucleolar volume was 6.88 $\mu m^3$. The diplotene nucleus had a volume of 124.8 $\mu m^3$ and a nucleolar volume of 16.37 $\mu m^3$.

*Pairing:* Half of the *alcoy* nuclei examined were in early prophase I stages. Figure 1 shows lateral components from the leptotene nucleus that was not reconstructed. Pairing appears to be in progress, but no complete synaptinemal complexes were visible in the sections of this nucleus. Juxtaposition of lateral components occurs, and an apparent central component nodule was observed (Figure 1) in a region where the minimum distance between lateral components is approximately 160 nm. Some telomeres were observed in association with the nuclear envelope. In an incompletely reconstructed zygotene nucleus, approximately 10% of the lateral components were paired into ten short stretches of synaptinemal complex at or near the nuclear envelope. Of the expected 26 telomeres, 24 were found associated with the nuclear envelope. Three presumptive nodules were noted, similar to that in Figure 1.

The *alcoy* nuclei that were completely reconstructed appear to represent successive stages in synapsis (Table 1). In the earliest (N242—Figure 7) pairing ranged from 2% in quadrivalent C to 50% in bivalent 7, and unpaired lateral components lay roughly parallel to their homologues. In quadrivalent C, the full translocation alignment has been accomplished even though only one short interstitial segment had paired to form a synaptinemal complex. There were nine nodules. In nucleus N340, pairing was 58% completed, and 14 nodules were found. Nucleus N230 (Figure 8) was 79% paired, only quadrivalent B having two arms largely unpaired. In both zygotene and pachytene nuclei, the ends of chromosomes (paired or unpaired) were distributed all round the nuclear envelope with no sign of clustering in any regions.

The early diplotene *asco* nucleus (N359), which was reconstructed, had 20 pieces of synaptinemal complex ranging up to 3.4 $\mu$m in length. Some unpaired lateral components were also visible, but continuous bivalents could not be reconstructed. Sixteen nodules were found in the regions of the synaptinemal complex which remained.




FIGURES 1 to 5.—Electron micrographs of Neurospora ascal nuclei. (1) shows a leptotene stage in which lateral components (paired arrows) are approaching each other. (N?) is a presumptive nodule and (NOR) is the nucleolus organizer region. (2a, 2b) are slanting longitudinal sections

TABLE 1

*Chromosome lengths in μm* (L) *number of nodules* (N) *and pairing* % (P) *in nuclei of* alcoy *a* × *Emerson A asci*

| Chromosomes | | N242‡ | N385 | N340 | Cell N219 | N230 | N388 | N333 | Mean ± SE | Estimated *N* value† |
|---|---|---|---|---|---|---|---|---|---|---|
| Quad. A | *L* | 16.1 | 17.8 | 18.0 | 17.7 | 16.1 | 19.75 | 18.7 | 18.0 ± 0.49 | |
| (1 + 6) | *N* | 5 | 5 | 9 | 4 | 8 | 5 | 6 | 6.2 ± 0.79 | 6.32 |
| | *P* | 30 | 40 | 75 | 80 | 100 | 80 | 100 | | |
| Quad. B | *L* | 13.9 | 15.3 | 14.1 | 18.9 | 14.6 | 13.25 | 19.8 | 16.0 ± 1.11 | |
| (2 + 4) | *N* | 2 | 5 | 2 | 4 | 2 | 4 | 6 | 3.8 ± 0.65 | 4.62 |
| | *P* | 15 | 50 | 45 | 46 | 45 | 95 | 100 | | |
| Quad. C | *L* | 9.6 | 13.1 | 11.55 | 12.0 | 11.15 | 13.55 | 17.1 | 13.1 ± 0.89 | |
| (3 + 5) | *N* | 0 | 4 | 2 | 4 | 5 | 4 | 3 | 3.7 ± 0.42 | 4.70 |
| | *P* | 2 | 37 | 30 | 100 | 80 | 100 | 65 | | |
| Biv. 7 | *L* | 5.6 | 4.15 | 4.8 | 7.0 | 6.2 | 6.7 | 5.1 | 5.65 ± 0.47 | |
| | *N* | 2 | 3 | 1 | 2 | 3 | 3 | 0 | 2.0 ± 0.52 | 2.24 |
| | *P* | 50 | 100 | 100 | 100 | 100 | 100 | 100 | | |
| Total | *L* | 45.2 | 50.35 | 48.45 | 55.6 | 48.05 | 53.25 | 60.7 | 52.7 ± 1.99 | |
| | *N* | 9 | 17 | 14 | 14 | 18 | 16 | 15 | 15.7 ± 0.67 | 17.86 |
| | *P* | 22 | 47 | 58 | 75 | 79 | 91 | 90 | | |

† Based on linkage map distance (PERKINS and BARRY 1977) and translocation identification of PERKINS *et al.* (1969).

‡ Not included in calculation of means (early zygotene ascus).

*Reconstructions:* The results of reconstructing eight *asco* nuclei at pachytene stage are given in Table 2. The mean proportional lengths of the seven chromosomes agree with previous values (PERKINS and BARRY 1977). The absolute lengths are closer to published light microscope pachytene lengths (MCCLINTOCK 1945; SINGLETON 1953) than to previous electron microscope estimates (GILLIES 1972), possibly because slightly later meiotic stages were used.

Serial sections of the interchange point in quadrivalent C of *alcoy* nucleus N219 are presented in Figure 6; here pairing is almost complete with little asynapsis around the interchange, and two nodules are present in these sections. However, pairing around the interchange point was variable and often incomplete (*cf.*, Figure 8).

It was usually possible to distinguish the three quadrivalents after reconstruction (Figures 7 and 8). Quadrivalent B has the nucleolus organizer region (NOR) close to one telomere (Figure 4). Quadrivalent A is about 50% greater in total length than quadrivalent C. Quadrivalent A is therefore presumed to

through a synaptinemal complex with a spindle shaped nodule (N). (3a, 3b) are serial sections through two synaptinemal complexes with nodules (Nx) and Ny). (4) shows a spindle shaped nodule (N) in the synaptinemal complex of chromosome *2* near the nucleolus organizer region (NOR). (5) shows a synaptinemal complex with three small nodules (arrows) within 1 μm. Figure (1) × 26,100; (2) × 19,400; (3) × 56,500; (4) × 19,400; (5) × 19,400.

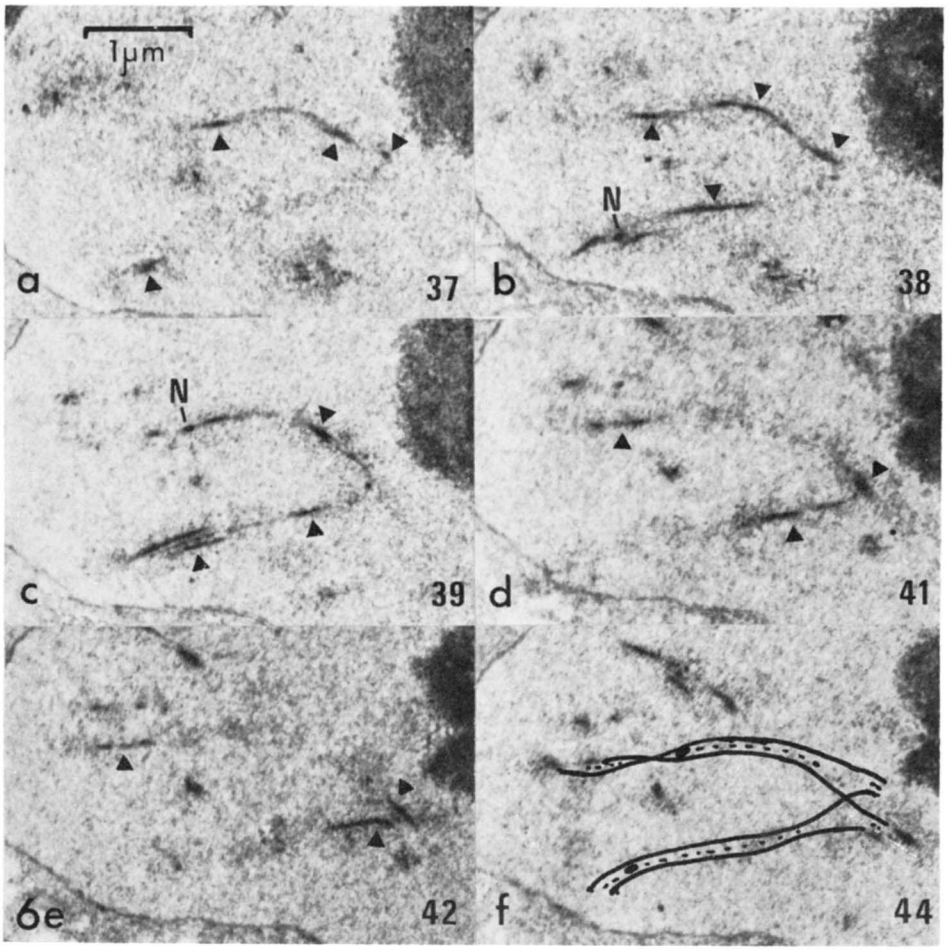



FIGURE 6.—Serial electron micrographs through the arm intersection region of the synaptinemal complex from quadrivalent C of cell N219. Section numbers are shown in right hand corners. Arrowheads point to the synaptinemal complex of the quadrivalent, and (N) indicates a nodule. In Figure 6f, a reconstruction of the quadrivalent in sections 37 to 44 is drawn. × 13,500.

be *T(I;II)*—chromosomes *1* and *6*; quadrivalent B is assumed to be *T(IV;V)*—chromosomes *4* and *2*; and quadrivalent C is assumed to be *T(III;VI)*—chromosomes *3* and *5* (PERKINS and BARRY 1977). Table 1 gives the lengths of chromosomes in the six early to mid-pachytene *alcoy* nuclei plus one zygotene nucleus. The mean total lengths of the three quadrivalents as proportions of total pachytene genome length were 0.341, 0.303 and 0.248, compared with expected values of 0.337, 0.298 and 0.268 based on the *asco* results, or 0.347, 0.297 and 0.265 based on light microscope data of MCCLINTOCK (1945) and SINGLETON (1953).

The mean *alcoy* genome length (52.7 μm) was shorter than the *asco* value, but the *alcoy* nuclei were predominantly zygotene stages. Haploid complement length in *alcoy* nuclei was positively correlated with percent pairing ($r = 0.72$). Chromosome *7* length was not significantly different in the *asco* and *alcoy* nuclei.

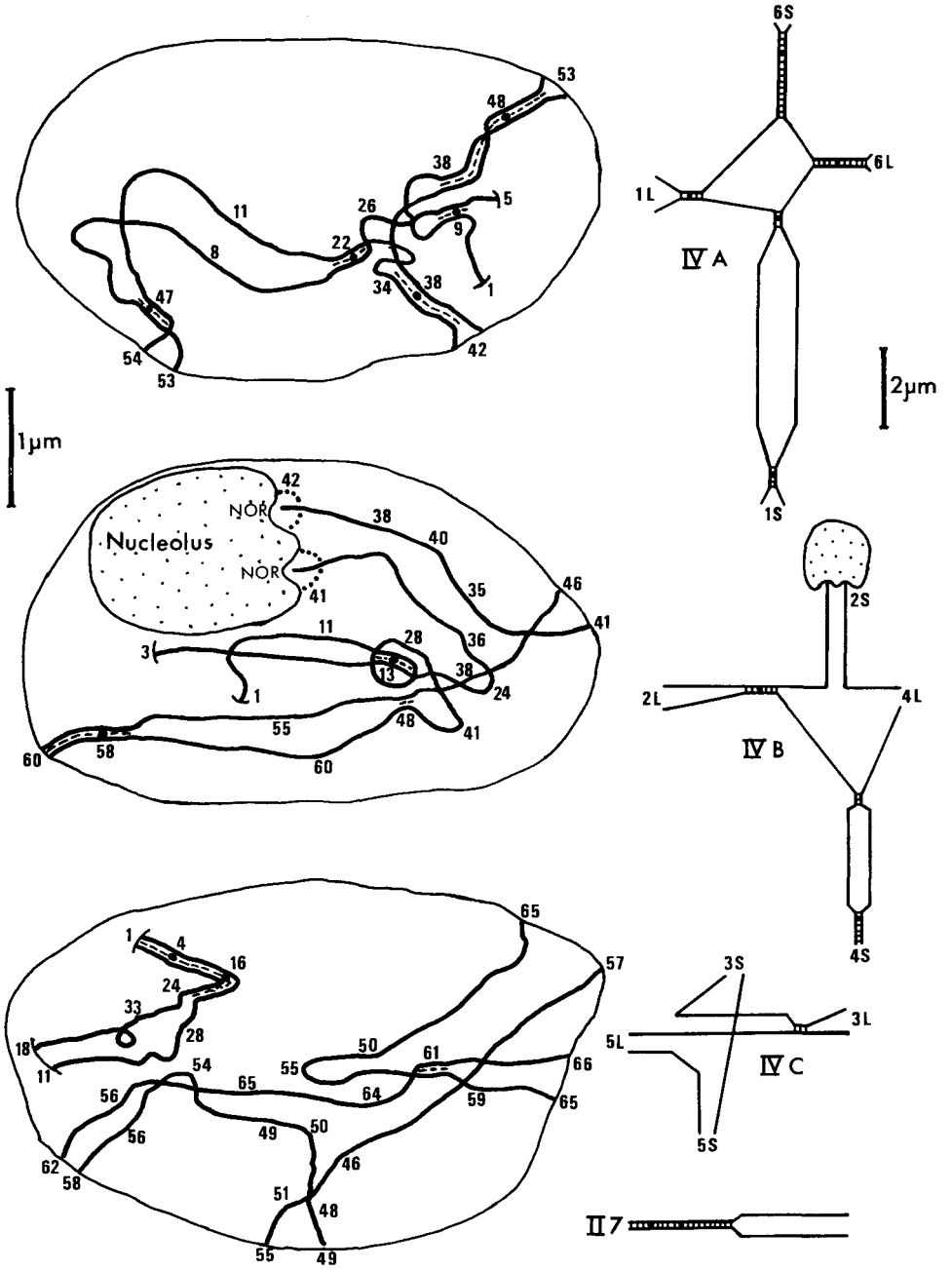



FIGURE 7.—Reconstructions of the three quadrivalents and one bivalent from zygotene stage *alcoy* nucleus N242. The figures on the left side are the tracings of the micrographs, with lateral components as heavy continuous lines, nuclear envelopes as thin lines, synaptinemal complex as dashed lines, and section numbers shown. The figures on the right side are diagrams in which lengths have been corrected for section thickness. Synaptonemal complex is indicated by cross bars between the lateral components, and the chromosome ends are identified. In tracings and diagrams nodules are indicated by heavy spots. Left side × 15,000; right side × 5,000.

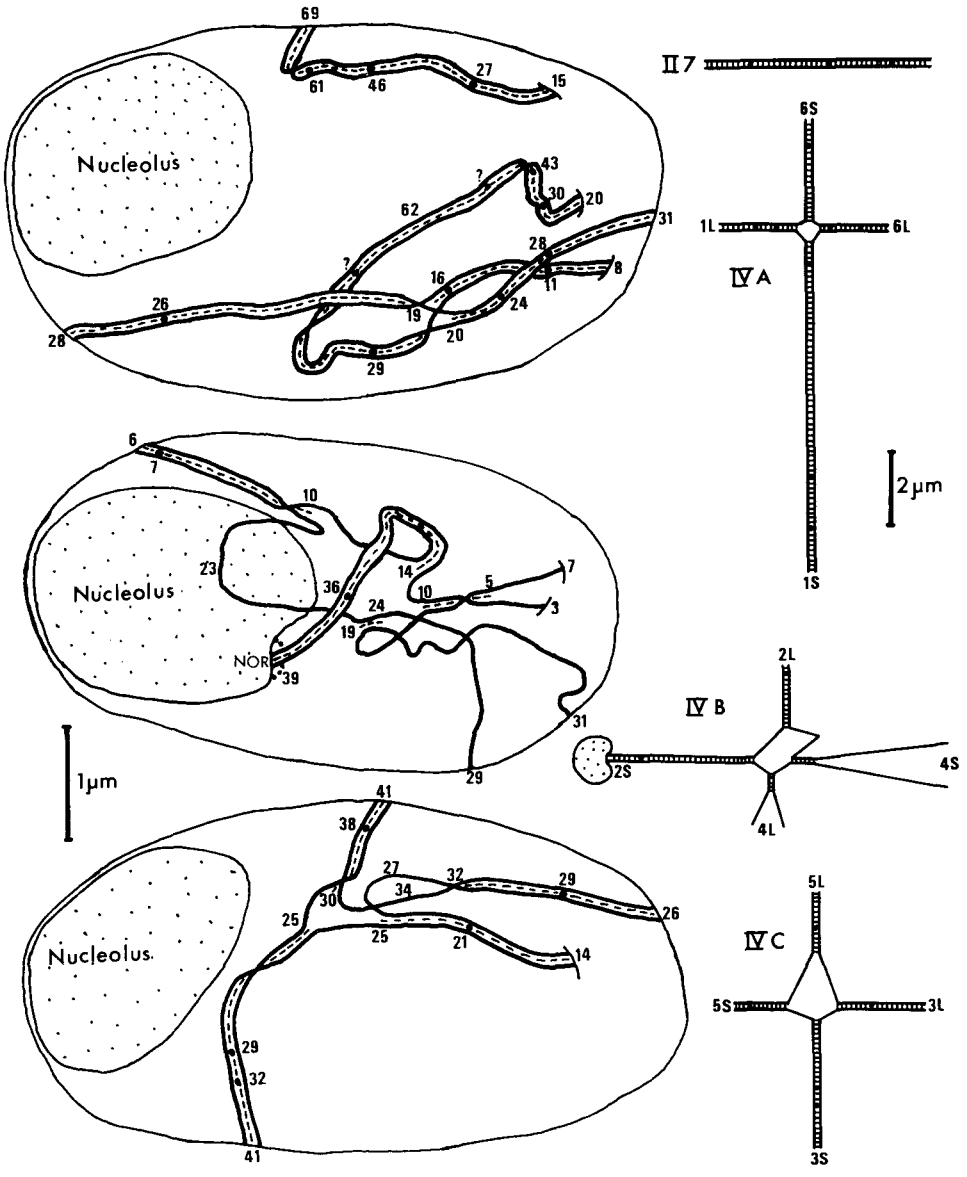



FIGURE 8.—Reconstruction of quadrivalents and bivalents from early pachytene stage *alcoy* nucleus N230. Notations and magnification as in Figure 7.

*Recombination nodules:* Central component nodules as previously described by GILLIES (1972) in *Neurospora* crassa are illustrated in Figures 3a and 3b. They are as dense as or denser than the lateral components, roughly oval in longitudinal section and circular in cross section. A considerable variation in size and shape of nodules was found, the previously described nodules representing only the larger end of the range. Other nodules were considered narrower

TABLE 2

*Chromosome lengths in μm* (L) *and number of nodules* (N) *in pachytene nuclei of* asco *a* × *Emerson A asci*

| Chrom. No. | | Cell A | B | C | D | E | F | G | H | Mean ± SE | Estimated values† |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *L* | 11.05 | 15.8 | 15.7 | 12.0 | 12.4 | 9.95 | 13.6 | 15.6 | 13.3 ± 0.80 | 15.0 |
| | *N* | 3 | 5 | 6 | 5 | 4 | 4 | 3 | 4 | 4.25± 0.37 | 4.00 |
| *2* | *L* | 9.9 | 7.6 | 11.75 | 9.1 | 8.6 | 7.75 | 12.1 | 8.95 | 9.5 ± 0.60 | 11.1 |
| | *N* | 2 | 1 | 1 | 2 | 5 | 5 | 2 | 3 | 2.63± 0.57 | 2.82 |
| *3* | *L* | 8.25 | 9.3 | 9.0 | 7.8 | 7.5 | 9.45 | 9.15 | 9.05 | 8.7 ± 0.26 | 8.7 |
| | *N* | 4 | 2 | 1 | 3 | 2 | 3 | 4 | 3 | 2.75± 0.37 | 1.88 |
| *4* | *L* | 7.05 | 9.2 | 8.95 | 6.8 | 7.3 | 6.95 | 7.9 | 8.8 | 7.9 ± 0.38 | 7.3 |
| | *N* | 4 | 2 | 2 | 2 | 3 | 5 | 2 | 2 | 2.75± 0.41 | 1.80 |
| *5* | *L* | 6.45 | 7.0 | 8.75 | 6.3 | 7.2 | 6.35 | 6.6 | 7.2 | 7.0 ± 0.28 | 7.3 |
| | *N* | 3 | 1 | 2 | 2 | 3 | 4 | 2 | 4 | 2.63± 0.38 | 2.82 |
| *6* | *L* | 6.15 | 6.05 | 8.15 | 5.7 | 6.9 | 5.3 | 6.35 | 6.4 | 6.4 ± 0.30 | 6.25 |
| | *N* | 1 | 2 | 3 | 2 | 3 | 4 | 3 | 2 | 2.50± 0.33 | 2.32 |
| *7* | *L* | 5.55 | 5.35 | 6.85 | 4.5 | 5.6 | 5.1 | 6.3 | 5.6 | 5.6 ± 0.25 | 5.6 |
| | *N* | 1 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 1.75± 0.25 | 2.24 |
| Total | *L* | 54.4 | 60.3 | 69.15 | 52.2 | 55.5 | 50.85 | 62.0 | 61.6 | 58.25± 2.17 | 61.25 |
| | *N* | 18 | 16 | 17 | 18 | 22 | 27 | 17 | 19 | 19.25± 1.28 | 17.86 |

† Lengths from McCLINTOCK (1945); nodule numbers calculated on basis of one nodule per 50 map units, using linkage map distances measured from Figure 5 of PERKINS and BARRY (1977). Linkage group I is 200 map units. Variation in crossing over in specific intervals (CATCHESIDE and CORCORAN 1973) will cause alterations in these values (see text).

in width (Figures 2, 4 and 5), and often difficult to see in cross sections and grazing longitudinal section (Figure 6c).

The nodules ranged in length from 55 to 210 nm, with a mean length of 136 ± 5.5 nm (Figure 9b), and in width from 20 to 90 nm, with a mean width of 55 ± 1.5 nm (Figure 9a). The length distribution (Figure 9b) shows a broad, almost bimodal, spread. There appeared to be a class of longer, spindle-shaped nodules, as well as the originally described oval shaped ones. In an attempt to document this, the distribution of length × width in 57 longitudinally sectioned nodules was plotted (Figure 10). There was a small overall positive correlation of width with length ($r = 0.32$, $p = 0.05$). No obvious separation of the points into two discrete populations could be made, although possibly two overlapping populations existed. In analyses of nodule number and position, all sizes of nodules were included.

In the *asco* nuclei, six cases were noted where two or three nodules (usually smaller in width) were found within 1 μm of synaptinemal complex (Figure 5).

In Table 2 the total number of nodules found in each of eight *asco* nuclei is shown. The mean total number is 19.25 ± 1.28 nodules per nucleus. In another

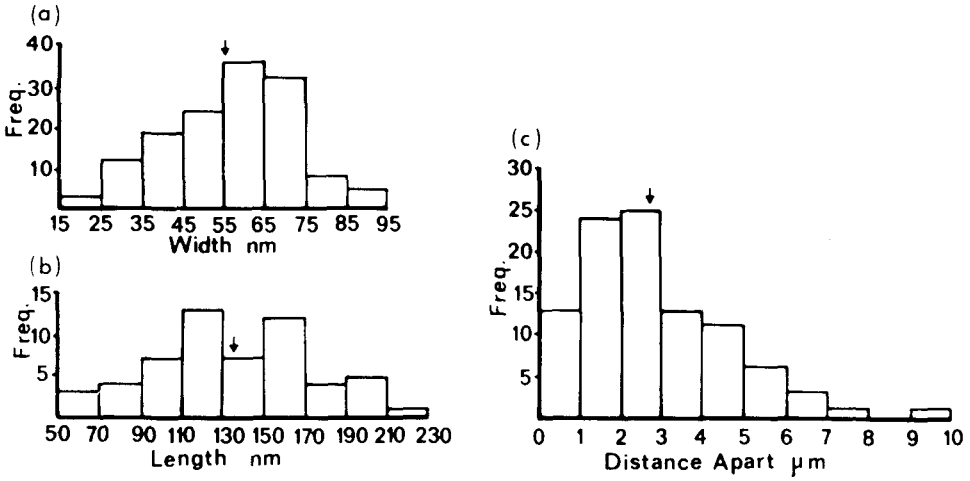



FIGURE 9.—Nodule size distribution and spacing. (9a) Distribution of widths of 109 nodules in *asco* and *alcoy* nuclei. Mean width 55 ± 1.5 nm, indicated by arrow. (9b) Distribution of lengths of 57 longitudinally sectioned nodules in *asco* and *alcoy* nuclei. Mean length 136 ± 5.5 nm, indicated by arrow. (9c) Distribution of distances between pairs of nodules within *asco* bivalents. Mean separation 2.76 ± 0.18 μm, indicated by arrow.

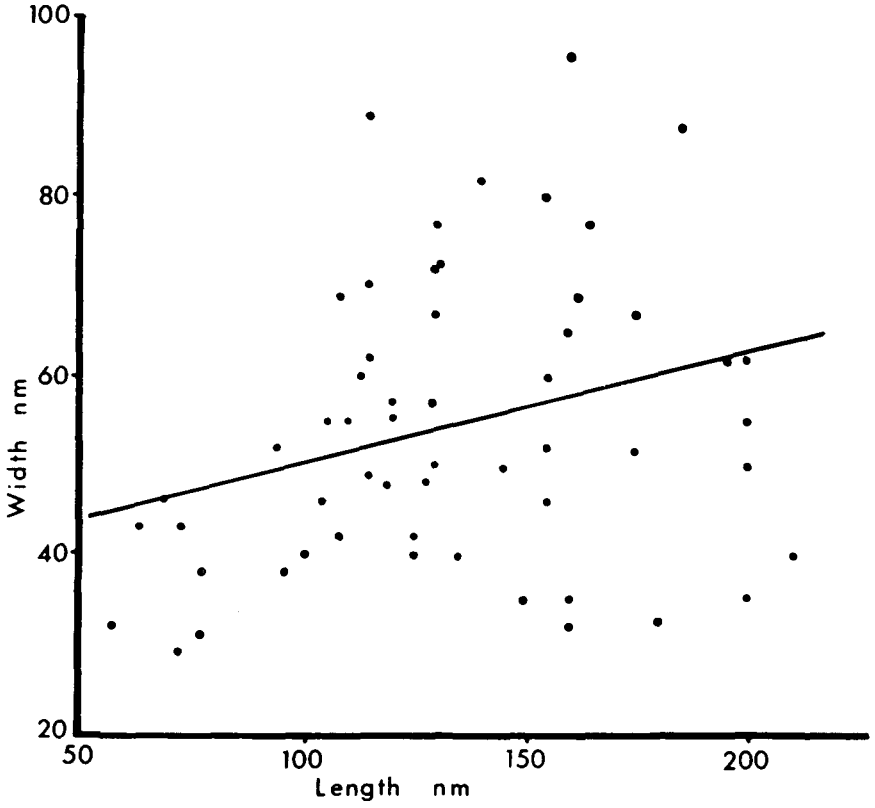



FIGURE 10.—Plot of length × width of 57 longitudinally sectioned nodules. The correlation coefficient is 0.32.

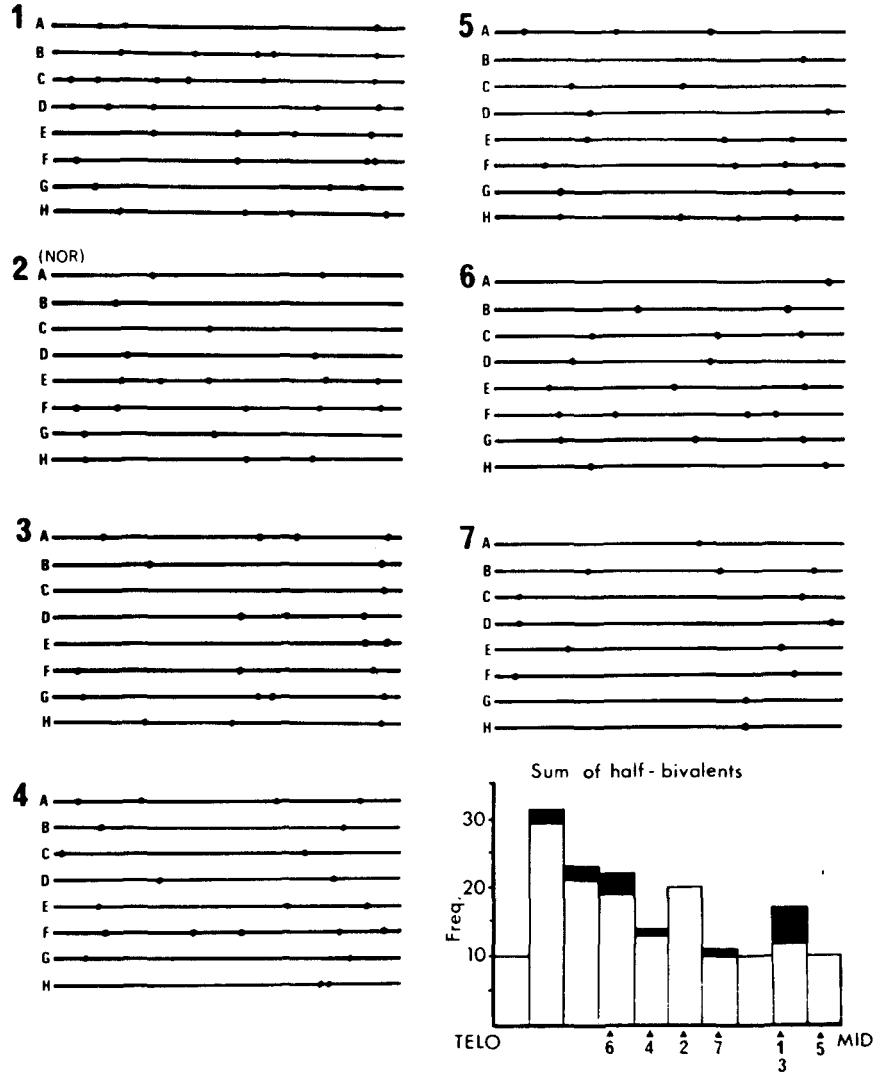



FIGURE 11.—Distribution of nodules in the seven bivalents of asco nuclei A to H. All bivalents have been standardized to one length. The NOR is at the left hand end of No. *2* bivalents. The bottom right histogram is the sum of the frequencies of occurrence of nodules in 10% segments of all half-bivalents, from the telomere on the left (TELO) to the middle of the bivalent on the right (MID). Additional nodule occurrences from *alcoy* No. 7 bivalents are in black. The numbered arrowheads indicate the mitotic metaphase centromere positions of the seven chromosomes from SINGLETON (1953).

five *asco* nuclei that could not be reconstructed because of missing sections, three nuclei had 12 nodules, one had 11 nodules and one had 14 nodules. These are necessarily minimum values as missing sections could have contained more nodules.

The numbers of nodules in the three quadrivalents and the bivalent of the *alcoy* nuclei are given in table 1. The mean total number was 15.7 ± 0.67 nodules

per nucleus. The bivalent number seven in *alcoy* nuclei had a mean of 2.0 nodules and in *asco* a mean of 1.75 nodules. These values were not significantly different. The early zygotene nucleus (N242, Figure 7) had nine nodules, and the diplotene nucleus (N359) had 16 nodules.

To compare the distribution along the bivalents of nodules in different nuclei, the lengths of all bivalents in the *asco* nuclei were normalized to 100 units, and the positions of nodules plotted for each bivalent. As centromeres are not distinguishable, it is not possible to differentiate the two telomeres of each bivalent (chromosome *2* is the exception because of the occurrence of the NOR near the short arm telomere). A biased assignment was made so that the maximum alignment of nodules close to one telomere occurred (*cf.*, ZICKLER 1977). The results for each bivalent in the eight *asco* nuclei reconstructed are given in Figure 11.

In an attempt to eliminate bias in assigning telomere alignments, each bivalent was divided in half, and the frequency of occurrence of nodules in 10% lengths of each half was noted from the telomere to the middle. The results from the two halves of all bivalents were summed and are presented as a histogram in Figure 11 (bottom right). The mitotic metaphase positions of centromeres as determined by SINGLETON (1953) are indicated. Additional nodule positions from seven *alcoy* bivalent 7's are included.

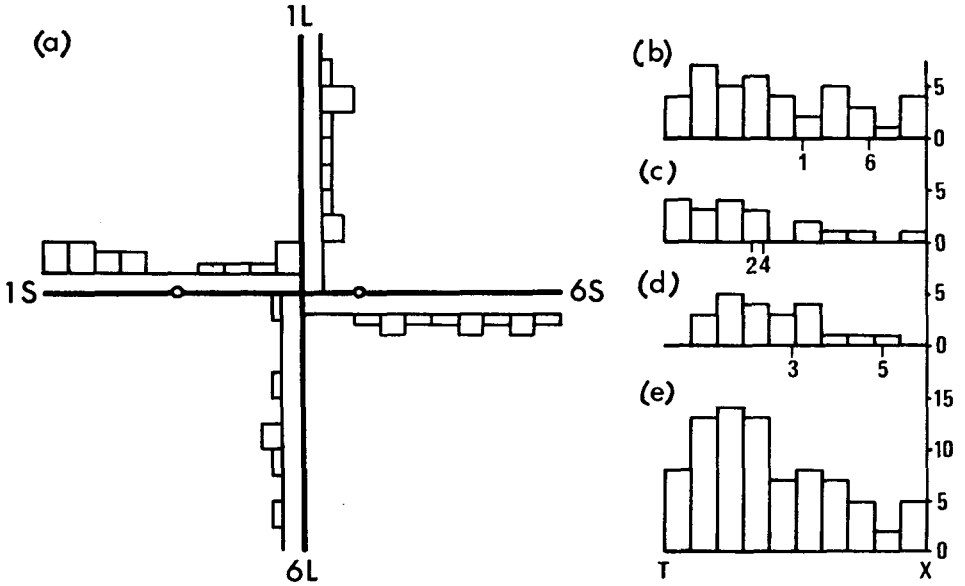



FIGURE 12.—Distribution of nodules in quadrivalents of *alcoy* nuclei. (12a) shows quadrivalent A with the four arms normalized to the same length, centromeres indicated by open circles, and long and short arms named. The histograms show frequencies of nodules per 10% segment of each quadrivalent arm. Histograms (12b-d) show the distribution for the sum of four arms of quadrivalent A, B, and C, respectively. Telomeric ends (T) on the left, arm intersection (X) on the right. Numbers under horizontal axes indicate centromere positions. Histogram (12e) is the sum of the data from all three quadrivalents. Frequencies of nodules appear on the right-hand vertical side.

The distance between nodules was measured for all *asco* nodules, and the results are presented in Figure 9c. The majority of nodules were closer together than 3 μm, with the mean separation of 97 pairs of nodules being 2.76 ± 0.18 μm.

An attempt was also made to analyze the nodule positions in the arms of the *alcoy* quadrivalents, although the sample (seven nuclei) was not large. In quadrivalent A (chromosomes *1* and *6*), the four arms are readily distinguished by length (see below). Figure 12a shows the distribution of nodules in the four arms of quadrivalent A normalized to the same length. The positions of the centromeres are indicated. Figure 12b shows the sum of the four arms of quadrivalent A, while Figures 12c and 12d show these sums for quadrivalents B and C, respectively. Finally, the data from all three quadrivalents is summed in Figure 12e. The positions of centromeres are indicated in Figures 12b, c and d. Centromere positions were estimated by converting linkage map distance (PERKINS *et al.* 1969) to pachytene distances, using the data from Table 2.

## DISCUSSION

The increase in Neurospora nuclear and nucleolar volume from leptotene to pachytene has been well documented in the light microscope (SINGLETON 1953). Recently, ZICKLER (1977) demonstrated a similar increase in Sordaria, which was accompanied by a 34% increase in chromosome length (as represented by lateral components). The data presented here from Neurospora show a highly significant positive correlation between nuclear diameter and haploid complement length ($r = 0.80$). Amongst the *alcoy* nuclei, the zygotene nucleus N242 (Figure 7) had a diameter of 4.9 μm and a haploid chromosome length of 45.2 μm. Four mid-zygotene nuclei (approximately 65% paired—N385, N340, N219, N240) had a mean nuclear diameter of 5.1 μm and a mean haploid genome length of 50.6 μm. The two early pachytene nuclei (N333, N388) had a mean diameter of 5.65 μm and a mean haploid genome length of 57.0 μm. The smallest *asco* nucleus (Cell F, 4.65 μm diameter) had the shortest of the *asco* chromosome components. The mean nuclei diameter of the eight *asco* nuclei in Table 2 was 5.5 μm.

The reconstructed translocation heterozygote quadrivalents from *alcoy* nuclei were readily classified by their chromosome length and arm proportions. Quadrivalent A had arms of 0.51, 0.22, 0.15 and 0.12 proportional length. BARRY (1967) published a light microscope picture of pachytene of *T(I;II)* 4637 *al*-1 from which arm proportions appear to be 0.44, 0.22, 0.18 and 0.16. The expected value based on the linkage map breakpoints (PERKINS *et al.* 1969) were 0.49, 0.23, 0.17 and 0.11, assuming a constant relationship in each chromosome between linkage distances and pachytene distances.

*Pairing*: In the earliest stage examined (leptotene, Figure 1) nucleolar fusion was complete, lateral components were apparently continuous and pairing was initiating. The first complete synaptinemal complexes appear at both interstitial and distal regions of the quadrivalents in N242. Distal pairing begins near, but not necessarily, at the nuclear envelope, in accord with findings in other species

(GILLIES 1975; HOLM 1977). Telomere ends at zygotene and pachytene were distributed around the nuclear envelope apparently in random order; no evidence of clustering of lateral components into a bouquet was noted. In the *alcoy* nuclei, pairing was always completed first in bivalent 7, suggesting that the difficulty of alignment in quadrivalents delayed synaptinemal complex formation. However, as no comparable data are available from *asco* nuclei, this may also be attributable to the shortness of bivalent 7. There appears to be a thickening of lateral components from their unpaired state at leptotene (Figure 1) to their appearance in synaptinemal complexes at pachytene (Figure 2).

*Recombination nodules:* The difference in dimensions of nodules (Figures 9a, 9b and 10) may represent differential developmental stages in the temporal life of the nodule. CARPENTER (1975) noted more variability of nodule size in younger Drosophila oocytes than in older cells. However it should be noted that the smaller central component nodules described here were also present in the nuclei with longest chromosomes (Table 2), which were presumably the oldest. Also, both large and small nodules were found in the early diplotene nucleus sectioned. The nodules appear quite early in the pairing process (Figure 1), and as can be seen in Figure 7, the occurrence of synaptinemal complex formation in one region of a quadrivalent at zygotene usually means the presence of a nodule in that region. It is possible that two functionally different types of nodules exist, although the evidence here from Neurospora is not conclusive. CARPENTER (personal communication) found small elipsoidal nodules in early pachytene stages of *Drosophila melanogaster* oocytes.

The mean total numbers of nodules present in the two Neurospora strains (Tables 1 and 2) were significantly different at the 5% level, presumably as a result of *alcoy* nuclei being at earlier stages. The number of nodules per bivalent or quadrivalent, and total number per nucleus, were compared with expected numbers, estimated on the basis of one nodule for every 50 map units of the linkage map of PERKINS and BARRY (1977). Linkage distancss were measured from Figure 5 of PERKINS and BARRY (1977), making allowances for scale changes, and letting linkage group I be 200 map units. Because *Neurospora crassa* strains differ widely with respect to their content of *rec* genes that regulate the level of recombination in specific regions (CATCHESIDE 1975), the linkage map distances are only an approximation of what can be expected in any particular cross. Although recombination may be reduced in specific intervals as a result of the presence of $rec^+$ genes (CATCHESIDE and COCHRAN 1973), it is not known if there are compensating increases elsewhere in the genome. There are no adequate studies that establish whether total recombination is altered by *rec* genes. PERKINS and BARRY (1977) consider that the map is a minimum of 700 map units (from chiasma counts), and probably is closer to 1000 map units.

In the *asco* nuclei, the mean numbers of nodules found were close to expectation in all bivalents and the genome total (Table 2). Only chromosome 3 showed a statistically significant variation from the estimated value ($t$ values are not shown owing to the unreliability of the estimated values on which they were calculated). In the *alcoy* nuclei, the three quadrivalents, the bivalents and the

genome total all had the expected number of nodules. There was no correlation of nodule number with nuclear diameter or total haploid genome length.

The number of nodules found agrees well with expectation if each nodule represents a crossover event. The number of nodules in *alcoy* nuclei is probably an underestimation, since some cells were not completely synapsed, and occasional small nodules may be missed in cross section. The agreement of nodule number with crossover frequency in Neurospora, in Sordaria (ZICKLER 1977) and in yeast (BYERS and GOETSCH 1975) adds to the evidence in support of CARPENTER's (1975) contention that nodules are recombination-specific structures. In the rat, MOENS (1978) has recently found that the number of nodules per nucleus (20) is about half the chiasma frequency. RASMUSSEN and HOLM (personal communication) have found approximately 75 nodules per male human pachytene nucleus.

Based on the total linkage map, there should be a crossover event approximately every 3$\mu$m in *Neurospora crassa*. From Table 2 it is possible to calculate that in the seven bivalents synaptinemal complex length per nodules varies from 2.55 $\mu$m in chromosome *6* to 3.61 $\mu$m in chromosome 2. The distance between nodules within bivalents (Figure 9c) may give an indication of interference distance. However, it should be remembered that centromeres may be included in many of these regions.

The analysis of the position of nodules in bivalents (Figure 11) and quadrivalents (Figure 12) show that there is evidence for localization of nodule positions. In the half-bivalent alignments (Figure 11, bottom right) it can be seen that nodules are localized in distal regions of chromosomes, the maximum number being in the region between 5% and 20% of the chromosome length from the telomere. The mitotic metaphase centromere positions shown should give a rough idea of pachytene centromere positions. If the largest heterochromatic chromomeres in Neurospora pachytene bivalents (PERKINS and BARRY 1977) represent the centromeric regions, distal localization of crossing over towards the telomeres might be expected, as found in many other organisms (MIKLOS and NANKIVELL 1976). However, bivalent *2* (Figure 11), the only case where the two telomeres are distinguishable, shows no evidence of a centromere effect. The data from the quadrivalents, while not very extensive (Figure 12), show a reduction in interstitial nodules, with a resultant distal localization of nodules. This agrees with SYBENGA's (1970) findings on chiasmata in rye translocation quadrivalents.

In Neurospora, then, the results show a tendency for median centromeres and distal localization of nodules. The number of nodules in Neurospora is in agreement with the expected number of crossover events. Thus, the limited evidence from Neurospora is consistent with that from Drosophila oocytes (CARPENTER 1975), namely that nodules in pachytene chromosomes agree in number and distribution with crossover events.

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