Meiosis in *Mesostoma ehrenbergii ehrenbergii*. IV. Recombination Nodules in Spermatocytes and a Test of the Correspondence of Late Recombination Nodules and Chiasmata

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Manuscript received July 7, 1988
Accepted for publication October 13, 1988

ABSTRACT

Male meiosis in *Mesostoma ehrenbergii ehrenbergii* (2x = 10) is characterized by extreme restriction of chiasma formation; 3 pairs of chromosomes form bivalents at metaphase I which are associated by single very distally localized chiasma, while two pairs of chromosomes remain as unpaired univalents. Electron microscopical three-dimensional reconstruction analysis of serial sections has been applied to 20 pachytene spermatocyte nuclei. In each nucleus three short stretches of synaptonemal complex (SC) were found, confined to a localized branched lobe of the nucleus, confirming the findings of an earlier study. The majority of reconstructed nuclei show that each of the three SC segments has a single prominent recombination nodule ("late" RN) associated with it. Late RNs in this system therefore show an excellent correspondence with metaphase I chiasmata, in contrast to a previous report. *M. e. ehrenbergii* is therefore not an exception to the hypothesis that meiotic exchange requires a functional late RN. A few nuclei had two, one or no RNs; these presumably represent nuclei that are not at the stage of maximum RN presence. Although *M. e. ehrenbergii* shows pronounced chiasma localization at the light microscope level, at the ultrastructural level RNs are widely distributed along the 5–10 μm of SC formed in each bivalent, indicating that genetic exchanges are not restricted to particular localized sites but occur at a large number of DNA sequences.

THE curious meiotic systems found in the oocytes and spermatocytes of the Rhabdocoel planarian worm *Mesostoma ehrenbergii ehrenbergii* (2x = 10) have a long history of investigation (SCHNEIDER 1883; VON VOSS 1914; VALKANOV 1938; HUSTED and RUEBUSH 1940; KEYL and GOLTENBOTH 1972; GOLTENBOTH 1973) and were recently the subject of detailed reinvestigation by light and electron microscopy (OAKLEY and JONES 1982; OAKLEY 1982, 1985). These studies confirmed previous observations that spermatocytes consistently form only three bivalents at metaphase I, each associated by a single very distal chiasma (OAKLEY and JONES 1982). The remaining four chromosomes are present as univalents which nevertheless regularly achieve accurate segregation at anaphase I (OAKLEY 1985). A parallel study of oocyte meiosis revealed that all five chromosome pairs exhibit achiasmatic association from diplotene through to metaphase I (OAKLEY 1982).

An earlier ultrastructural investigation of mid-prophase I spermatocytes, involving three-dimensional reconstruction of six nuclei, showed that synaptonemal complex (SC) formation and hence chromosome pairing is restricted to three short stretches occupying a lobed region of the nucleus (OAKLEY and JONES 1982). In other words, chromosome pairing in spermatocytes of this species is constitutively incomplete. It is deduced that pairing is limited to the ends of three chromosome pairs which are presumed to correspond to the three chiasmatically associated bivalent ends in spermatocyte first metaphases.

This unusual and interesting system presents an excellent opportunity to test the proposed correspondence between recombination nodules and classical chiasmata. Recombination nodules (RNs) are dense spherical or ellipsoid structures, about 100 nm in diameter, which occur in close association with the central elements of SCs in a wide range of eukaryotes (reviewed by VON WETTSTEIN, RASMUSSEN and HOLM 1984). Their role as structures mediating meiotic recombination was first proposed by CARPENTER (1975) and has received considerable support from other investigations across a wide range of organisms (reviewed by CARPENTER 1979b; VON WETTSTEIN, RASMUSSEN and HOLM 1984). More recently it has been proposed that two types of RNs exist which differ in their chronology of appearance (early *vs.* late), in their frequencies and distributions, and generally if not always in their morphologies (RASMUSSEN and HOLM 1978; CARPENTER 1979b). Early RNs are confined to zygotene in some species, but are retained into early pachytene in others. They have been suggested to have a role in promoting homologous pairing during zygotene (ALBINI and JONES 1987; CARPENTER 1987) and they may also mediate gene conversion at that time (RASMUSSEN and HOLM 1978; CARPENTER 1979a, 1987). Late RNs are restricted to pachytene and they are hypothesized to be involved

in some or all aspects of crossing over (reciprocal recombination, exchange) during pachytene. This hypothesis is based on the close correspondences which have been observed between the numbers and/or distributions of late RNs and crossovers or cytological chiasmata in a wide variety of species (CARPENTER 1979b; VON WETTSTEIN, RASMUSSEN and HOLM 1984). However, deficiencies of late RN numbers compared to chiasmata/crossovers have been reported in many species (ALBINI and JONES 1988), from which it is inferred that late RNs are ephemeral structures in these species; that is they are present only transiently during pachytene and consequently the full complement of late RNs may not be present simultaneously.

Applied to *M. e. ehrenbergii* male meiosis, the late RN hypothesis predicts that each of the three short SC stretches should contain a single late RN at some time during the progression of the cell through pachytene, since each of the bivalents has one chiasma at metaphase I. Because of the relatively short lengths of these SCs, this prediction should be relatively easy to test. Although six nuclei were thoroughly examined in the earlier study by OAKLEY and JONES (1982), RNs were not seen. Since this, if confirmed, would at least provide an exception to the so-far universal observation that a meiotic exchange requires a functional late RN, a further careful search for RNs was considered worthwhile.

MATERIALS AND METHODS

Culture conditions: Laboratory cultures of *M. e. ehrenbergii* were raised from dormant winter eggs which had been cold-treated (4° for at least 12 weeks) to overcome their natural diapause. Young animals were kept in filtered pond water and fed on brine shrimps which had been washed to remove all traces of brine. The animals were used when about 4 weeks old.

EM preparation: Whole animals were fixed in 4% glutaraldehyde in phosphate buffer (pH 7.2) for 4 hr at room temperature. The material was post-fixed in 2% osmium tetroxide in phosphate buffer (pH 7.2) for 2 hr and then dehydrated through an alcohol series. Some animals were stained with phosphotungstic acid (PTA), in which case the osmium tetroxide step was omitted and entire animals were treated with 1% alcoholic PTA at 20° for 10–16 hr after the alcohol dehydration series and before embedding. Entire animals were embedded in Epon 812 which was polymerized at 60° for 20 hr. Section series were cut from four different animals (see Table 1 for details).

Serial sectioning: Sections 100 nm thick were cut on a Reichert-Jung ultramicrotome equipped with a Diatome diamond knife. Ribbons of sections were transferred to Formvar-coated slot grids using the indirect method of Wells (1974). Sections from osmium-fixed animals were stained with 1% aqueous uranyl acetate at 40° for 90 min, followed by lead citrate at 20° for 2 min. The staining procedure was carried out in an automatic stainer (LKB 2168 Ultrostainer) to reduce the risk of stain deposits obscuring critical parts of sections.

Section series were examined in a JEOL 1200EX electron microscope and selected nuclei were photographed at magnifications of 6,000–7,500. SCs and RNs were traced from enlarged photographic prints onto acetate sheets and SC lengths and RN positions were measured using a Summagraphics digitizer linked to a BBC microcomputer which was programmed to allow for section thickness in calculating true SC lengths and RN positions.

RESULTS

Testis organization: *M. e. ehrenbergii* has a pair of long lobulated testes which lie along each side of the pharynx and vitelline glands. In adult animals the testes occupy about half the body length and make up roughly 25% of body volume. There is no obvious sequential arrangement of spermatogenic stages in the testis. Although cells at the same stage of spermatogenic development are grouped together in synchronized clusters, groups of cells at different stages bear no apparent relation to each other and occur scattered throughout the testis in an apparently unordered arrangement (OAKLEY and JONES 1982). Spermatogenic cells containing SC are morphologically distinct from other cell types in terms of nuclear shape and chromatin condensation as well as their possession of SC. These so-called "SC cells" are regarded as a developmental substage of a single population of spermatogenic prophase I cells. They contain roughly spherical or ellipsoidal nuclei but with a characteristic branched lobe arising from a localized part of each nucleus (Figure 1). The main body of each nucleus is 14–16 μm in diameter, and the lobe projects 6–8 μm from the main nucleus.

Synaptonemal complexes: Twenty nuclei containing SC were reconstructed from serial sections. In every case the SCs are confined to the projecting lobed region and the main body of each nucleus is entirely devoid of SC except for a limited zone at the base of the lobe. The morphology of the SC is somewhat atypical in this species. Its most striking feature is the prominent pale central region containing a very dense central element (Figure 2). The lateral elements flanking the central region are indistinct and their density closely matches that of the adjoining chromatin.

Each reconstructed nucleus was found to contain just three short SC stretches, as previously described by OAKLEY and JONES (1982). Each SC occupies one branch of the lobed region and terminates distally against the nuclear envelope at or near the closed end of one branch of the lobed region (Figure 2a). The nuclear envelope appears to be specialized at the point of SC attachment. This takes the form of darkly staining material occupying the space between the inner and outer nuclear membranes, which at higher magnification resolves into a number of dots. The invagination of the nuclear envelope at the point of SC attachment, visible in Figure 2a, is not a general feature of all SCs but occurs sporadically in about half

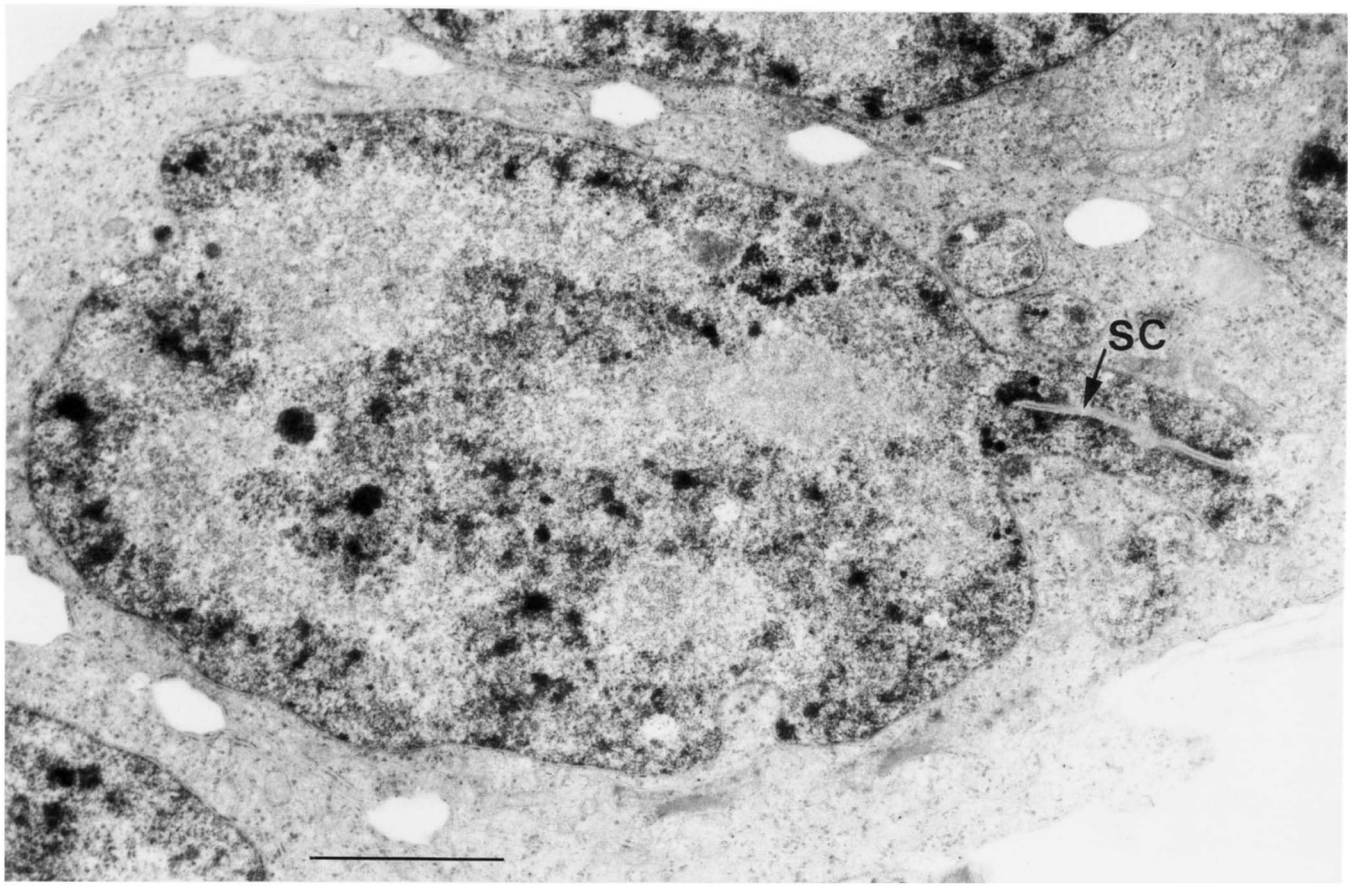

FIGURE 1.—A survey low power electron micrograph of an entire spermatocyte nuclear section showing SC confined to a lobe projecting from the main body of the nucleus (bar = 3 μm).

the SC attachments observed. The type of SC attachment is not consistent among the three SC ends in each nucleus, nor does it seem to be a feature of one particular telomere. Proximally the SCs end abruptly at or near the junction of the lobed region with the main body of the nucleus. The poor differentiation of the lateral elements against the surrounding chromatin make it impossible to decide whether they continue as unpaired axial cores into the main body of the nucleus. Consequently, the unsynapsed chromosomes cannot be traced by electron microscopy and, moreover, they are intractable at the light microscope level too.

Measurements of the SC lengths from 20 reconstructed nuclei are presented in Table 1. The average SC length is 6.85 μm (range 4.99–10.69 μm) and the average combined SC length per nucleus is 20.54 μm (range 16.65–29.11 μm). OAKLEY and JONES (1982) studying a smaller sample of nuclei found that the ranges of the ranked SC lengths did not overlap, and tentatively suggested that the three SC length ranks might correspond to three different individual bivalents. When the ranked relative lengths of SCs from the present study are plotted, an interesting division into two sub-sets of nuclei appears (Figure 3). In one set (1) the relative SC lengths are unique and nonoverlapping, the intervals between the 3 ranks being more or less equal. The other set (2) shows a very different pattern; one SC is much longer than the other two and the shorter SCs show similar and overlapping relative length distributions. The origin or cause of these two categories of nuclei is not obvious. The difference cannot be simply ascribed to inter-individual variation which might have an underlying genetic basis because three of the four animals studied included nuclei of both types. Some of the reconstructed nuclei are known to belong to synchronized clusters of cells within the same testis lobe. All four nuclei from one animal (B) belong to a single cluster and all of them are of type 2, which suggests that local developmental factors might be responsible. However, this degree of uniformity is not observed consistently. For example 3 adjacent nuclei from animal L, which probably belong to a single cluster, include two type 1 nuclei and one type 2 nucleus. Unfortunately information on spatial relationships is unavailable for most (10/13) of the nuclei from animal H. In the absence of distinctive markers identifying particular SCs, it is questionable whether different bivalents are consistently represented by SCs of different lengths. However, this remains a possibility, particularly in the case of the single longer SC observed in a sub-set of the reconstructed nuclei.

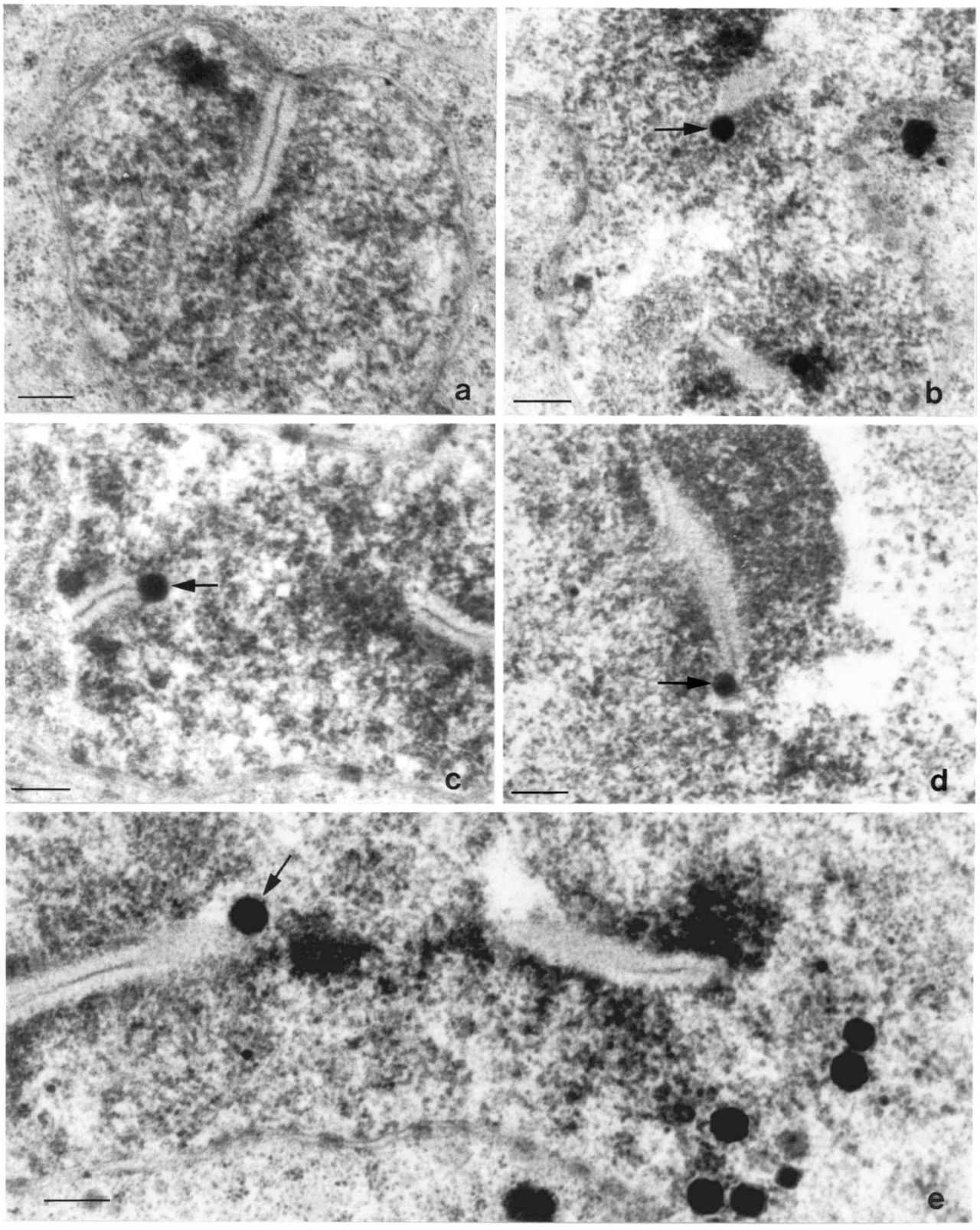

FIGURE 2.—Higher power electron micrographs of SCs and RNs. a, A terminal stretch of SC attached to the nuclear membrane at the distal end of a lobe. b–e, Different views of RNs (arrowed) in association with SCs; note especially the close association of RNs with the central element in sagittal sections (b and e). A group of SNBs is present in e (lower right), for comparison with the RN (bars = 0.25 μm).

TABLE 1

Total SC lengths per nucleus and individual SC lengths for 20 reconstructed nuclei

Nucleus	Total SC lengths (μm)	Individual SC lengths (μm)	RN positions (μm)
1 (H)	16.647	6.168 (0.371)	5.723 (0.927)
		5.491 (0.330)	2.848 (0.519)
		4.988 (0.299)	2.891 (0.592)
2 (L)	17.760	7.105 (0.400)	5.471 (0.770)
		5.405 (0.304)	1.043 (0.193)
		5.250 (0.296)	4.533 (0.863)
3 (D)	17.839	7.313 (0.410)	6.311 (0.863)
		5.449 (0.305)	2.017 (0.370)
		5.077 (0.285)	2.650 (0.522)
4 (H)	18.539	7.086 (0.382)	5.818 (0.821)
		6.424 (0.347)	1.053 (0.164)
		5.029 (0.271)	3.709 (0.738)
5 (H)	18.874	7.694 (0.408)	3.719 (0.483)
		5.649 (0.299)	
		5.531 (0.293)	
6 (B)	19.110	7.024 (0.368)	
		6.254 (0.327)	
		5.832 (0.305)	
7 (B)	19.222	7.685 (0.400)	0.593 (0.077)
		5.814 (0.302)	3.450 (0.593)
		5.723 (0.298)	4.872 (0.851)
8 (H)	19.546	7.070 (0.362)	1.815 (0.257)
		6.548 (0.335)	4.114 (0.628)
		5.928 (0.303)	4.325 (0.730)
9 (H)	19.696	7.256 (0.368)	4.225 (0.582)
		6.318 (0.321)	0.985 (0.156)
		6.122 (0.311)	3.276 (0.535)
10 (B)	19.980	7.807 (0.391)	2.924 (0.375)
		6.180 (0.309)	1.890 (0.306)
		5.993 (0.300)	4.682 (0.781)
11 (L)	19.981	7.386 (0.370)	6.788 (0.919)
		6.567 (0.329)	5.125 (0.780)
		6.028 (0.301)	5.883 (0.976)
12 (H)	20.096	7.387 (0.368)	4.651 (0.630)
		6.735 (0.336)	4.056 (0.601)
		5.956 (0.296)	
13 (B)	20.765	8.395 (0.404)	5.558 (0.662)
		6.340 (0.305)	2.108 (0.332)
		6.030 (0.290)	5.443 (0.903)
14 (H)	21.061	8.634 (0.410)	2.024 (0.234)
		6.554 (0.311)	
		5.873 (0.279)	
15 (H)	21.451	8.204 (0.382)	5.180 (0.631)
		7.113 (0.332)	4.561 (0.641)
		6.134 (0.286)	1.646 (0.268)
16 (L)	21.576	7.805 (0.362)	4.354 (0.558)
		7.150 (0.331)	3.115 (0.436)
		6.621 (0.307)	2.588 (0.391)
17 (H)	22.129	9.316 (0.421)	
		6.612 (0.299)	
		6.201 (0.280)	
18 (H)	22.155	9.490 (0.428)	4.155 (0.438)
		6.549 (0.296)	5.106 (0.780)
		6.116 (0.276)	0.400 (0.065)
19 (D)	25.314	10.032 (0.396)	7.929 (0.790)
		7.782 (0.307)	3.001 (0.386)
		7.500 (0.296)	2.493 (0.332)
20 (H)	29.109	10.689 (0.367)	4.669 (0.437)
		10.134 (0.348)	6.992 (0.690)
		8.286 (0.285)	5.342 (0.645)

Four different animals (B, D, H and L) are included in the study. Relative SC lengths as proportions of nuclear totals are given in parentheses. RN positions are expressed as absolute distances from the attached telomeric SC ends and relative distances (in parentheses) as proportions of entire SC lengths.

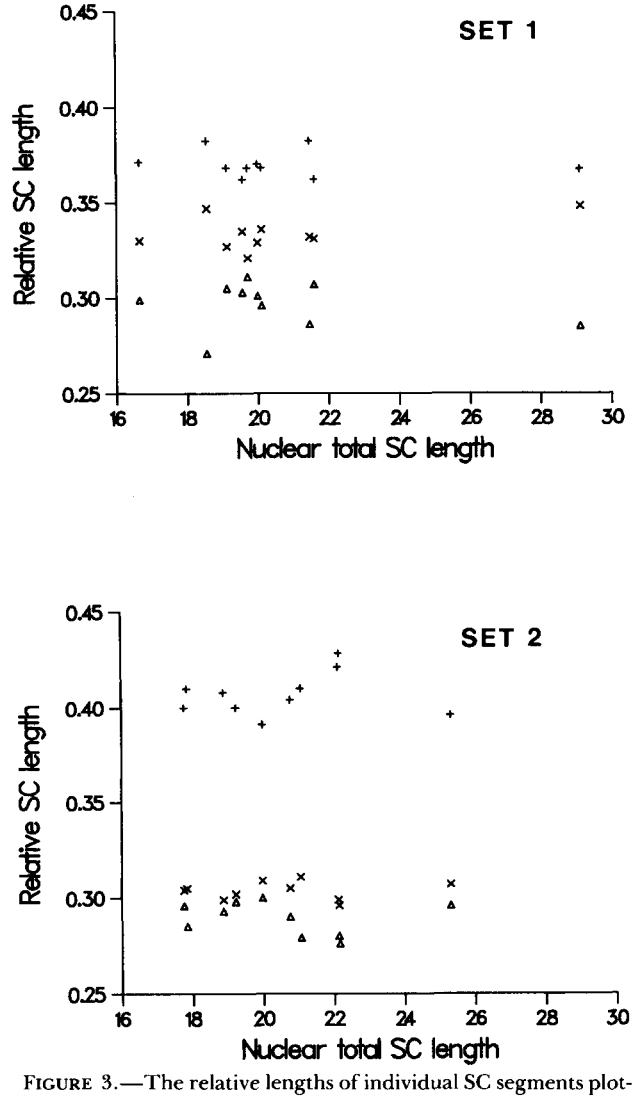

FIGURE 3.—The relative lengths of individual SC segments plotted against nuclear total SC lengths for type 1 and type 2 nuclei (see text for explanation). The longest (+), intermediate (×) and shortest (Δ) SC segments in each nucleus are indicated.

The total extent of SC in the nuclei studied varied between 16.55 and 29.11 μm. However, in all cases, the SCs extended continuously through the lobed region and ended abruptly at or very near to the convergence of the lobed region with the main body of the nucleus. Because pairing is constitutively incomplete in this system it is difficult, if not impossible, to demarcate zygotene from pachytene substages in prophase I. This is particularly so if synapsis proceeds sequentially, without discontinuities, from the attached telomeres. Nuclei with lobes but devoid of SC were not observed, which makes it likely that the lobes develop as synapsis proceeds. The limited range of SC lengths in these nuclei (16.65–29.11 μm) and the absence of nuclei with *less than* 16.65 μm of SC suggest that they constitute a relatively homogeneous population which are collectively at or close to the stage of maximum pairing, that is, pachytene. However, it cannot be excluded that synapsis may still be progress-

ing in some of the nuclei, though if that were the case pairing must be nearing completion. No conscious effort was made to exclude nuclei at earlier stages of pairing, but it is likely that the sampling method, which relied on a combination of general nuclear morphology, presence of lobes and detection of SC, would bias the nuclei sampled in favor of those with longer SCs. Early and mid-zygotene nuclei with shorter SCs would be less likely to be detected, particularly if they are also relatively scarce, reflecting a brief duration of the zygotene stage.

RNs: Uniform and very distinctive structures conforming to the general definition of RNs (CARPENTER 1975, 1979b) were consistently observed in most of the reconstructed nuclei stained with uranyl acetate and lead, but none were observed after PTA staining. The RNs of *M. e. ehrenbergii* are spherical densely stained bodies about 100 nm in diameter (Figure 2, b–e). and c). The great majority of RNs are located directly over the central element of the SC and span the entire width of the central region. Sections which cut through SCs in the sagittal plane [see Figure 4s in CARPENTER (1979a) for explanation] clearly show that RNs are located directly *on* the outer surface of the central element (Figure 2, b and e).

The identification of RNs in this species is complicated by the occurrence of a further class of dense spherical nuclear structures termed spherical nuclear bodies (SNBs). In general appearance these structures superficially resemble RNs but they are usually more densely staining with uranyl acetate and lead, they are delimited by a sharper boundary and they are more variable in size (Figure 2e). SNBs are much more numerous than RNs and they occur throughout the nucleus including the nonlobed region. Although SNBs are preferentially associated with chromatin, they are not closely associated with SCs. A quantitative study of the distribution of SNBs showed that they very rarely approach within 400 nm of SCs and the majority occupy peripheral locations on the outside of the chromatin which surrounds SCs. Consequently RNs can be confidently distinguished from SNBs despite their superficial resemblance. SNBs often occur in clusters, and although several nuclei (10/20) contain single clusters of at least four SNBs which are associated with the chromatin around one SC, there is no consistent pattern of association with SCs of particular ranked lengths. SNBs were not observed following PTA staining, either in the present study or in the earlier study by OAKLEY and JONES (1982).

Data on the occurrence and the positions of RNs on SCs are presented in Table 1. Of the 20 reconstructed nuclei, 15 contain 3 clear RNs. In each case, 1 RN is associated with each of the 3 SCs. A further 3 nuclei have either 1 RN (2 nuclei) or 2 RNs (1 nucleus) and 2 nuclei lack RNs entirely. In total therefore 49/60 of the reconstructed SCs contain a single clear RN and no SCs contain more than 1 RN. Interestingly, both nuclei that have only 1 RN have it on the longest SC and the single nucleus with 2 RNs has one on the longest SC and one on the SC of intermediate length. RN positions along SCs (Table 1) are expressed as distances from the attached SC ends, since this is the only constant reference point which is common to all SCs. As there are no clear grounds for identifying the 3 SC length ranks with different bivalents, the data on RN positions have been pooled over nuclei, and SCs within nuclei, before plotting in histogram form (Figure 4).

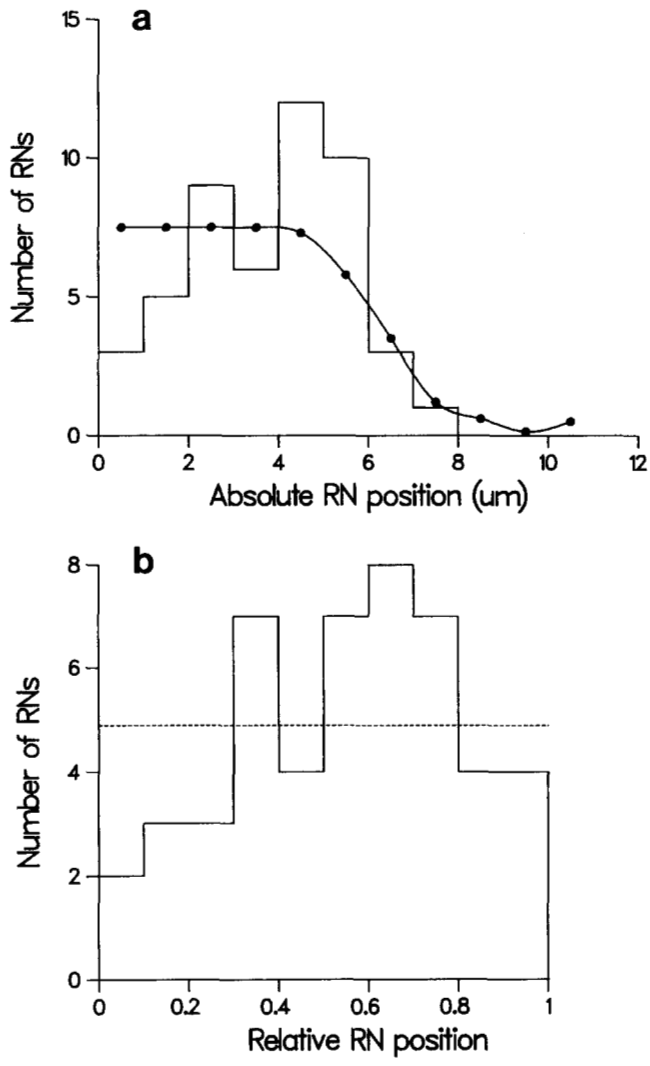

FIGURE 4.—Histograms showing the distribution of late RNs along the SCs of partially paired bivalents. a, Histogram plots of the distribution of absolute RN positions, measured from the attached SC ends, and also the expected distribution based on a uniform probability of RN occurrence along SCs (solid circles). b, Histogram of the distribution of relative RN positions, expressed as proportions of total SC lengths, and also the distribution of relative RN positions expected if RNs are uniformly distributed along SCs (broken lines).

RN positions, as absolute distances (μm) from the attached SC ends, are presented in Figure 4a, together

with the numbers of RNs expected in each 1-μm interval assuming a uniform probability of RN occurrence along the SCs. The latter values are based on the overall average RN frequency per 1 μm of SC (0.153) and the observed numbers of SCs of different lengths. Figure 4a shows that the RNs are widely but not uniformly distributed along SCs. There is a distinct peak of RNs in the 4–5-μm interval, with the RN frequency falling off sharply on either side. Comparison with the expected RN distribution reveals that there is a marked excess of RNs over the expected numbers in the 4–6-μm intervals and a marked deficiency in the 0–2-μm intervals. Only one RN appears in the 7–8-μm interval and none occur more distally although there are eight SCs between 8 and 11 μm long. In order to remove the effects of variation in absolute SC length, which could reflect different pairing extents or stage-related bivalent contraction, RN positions are also expressed as relative distances from the attached SC ends (Figure 4b). This also reveals that RNs are widely but unevenly distributed along SCs. Compared to the distribution expected if relative RN position is uniform along SCs, the observed distribution shows an excess, mainly between 0.5 and 0.8 of relative length, that is, skewed towards the unattached SC ends. Again there is a deficiency of RNs observed in the proximal region between 0 and 0.3 relative length.

DISCUSSION

The general pattern of meiotic pairing observed in this study of *M. e. ehrenbergii* spermatocytes confirms the findings of the previous investigation by OAKLEY and JONES (1982). SC formation is incomplete and limited to relatively short distal segments of 3 bivalents. However, the average total SC length per nucleus in the present study (20.54 μm) considerably exceeds that observed by OAKLEY and JONES (10.94 μm). It is unlikely that this difference is due to technical errors of measurement or calibration since the nuclear diameters coincide very closely in the two studies. Differences in SC extent between the two samples could possibly reflect stage differences. Alternatively, the final extent of SC formation may vary between different individuals, perhaps due to genetical causes. OAKLEY (1982) found that the total extent of SC formation in oocytes, which are almost fully paired with only short discontinuities of SC, was in excess of 400 μm.

Clear RNs were only observed during the present study after staining with uranyl acetate and lead citrate (UA/Pb). Ten PTA stained nuclei were thoroughly examined but rather surprisingly they did not reveal RNs although PTA has been shown to have a strong affinity for RNs in other organisms (*e.g.*, BERNELOT-MOENS and MOENS 1986; JENKINS and SCANLON 1987). The failure to detect RNs in the previous study of *M. e. ehrenbergii* is understandable in view of these findings, since it was mainly based on 6 PTA stained reconstructed nuclei although some casual observations were also made on UA/Pb stained survey sections.

The majority (15/20) of the nuclei in this study show a complete correspondence between RNs and metaphase I chiasmata. Each SC stretch has one and only one RN, and the three bivalents at metaphase I have one and only one chiasma. This correspondence suggests that these RNs are the *M. e. ehrenbergii* representatives of the category of "late RNs" as proposed by CARPENTER (1987), and this species is therefore not an exception to the general rule of one late RN being associated with one exchange event. In all other systems, late RNs have been observed during pachytene; although *M. e. ehrenbergii* lacks a classical pachytene stage, there is no reason to doubt that these nuclei are in the equivalent stage to pachytene for this species.

Three nuclei had either one or two RNs and two nuclei had no detectable RNs. Deficits of late RNs compared to chiasmata or genetical crossovers have been commonly reported (*e.g.*, GILLIES 1983; BERNELOT-MOENS and MOENS 1986; ALBINI and JONES 1988) but in general they are not regarded as invalidating the conclusion that late RNs are specifically associated with recombination events. Technical causes have been considered for RN deficits both in sectioned and spread material (STACK and ANDERSON 1986; ALBINI and JONES 1988). The favored explanation in most cases is transience of RNs during pachytene, so that none or only a proportion of all the recombination-associated RNs may be present during some substages of pachytene (CARPENTER 1975; GILLIES 1983; BERNELOT-MOENS and MOENS 1986; ALBINI and JONES 1988). In some systems the maximum number of late RNs are present at the same time in a proportion of nuclei (CARPENTER 1975; STACK and ANDERSON 1986) as in the present study. Accordingly, the occasional absence of RNs from SCs in the present study suggests some degree of RN transience in spermatocytes of *M. e. ehrenbergii*. The observation that nuclei with fewer than three RNs have them confined to the longest SCs in those nuclei suggests that RN transience may be in some way related to SC development.

A striking characteristic of the *M. e. ehrenbergii* meiotic system is that the three bivalents are limited to one and only one chiasma, yet each of the three bivalents makes an appreciable length of SC (roughly 5–10 μm). The regulation of meiotic exchange production may include a step requiring some minimal length of homologous SC in order to generate an exchange event (JONES 1984). If this is a general property which applies to *M. e. ehrenbergii* as well as

to other species, then the present data gives 5 μm (the shortest SC segment that has a RN) as the lower limit for this minimum length in *M. e. ehrenbergii*. However, if this concept of "minimum SC length" has general validity then the minimum length must vary between species since longer SCs are associated with obligate localized chiasmata in *Stethophyma grossum* (FLETCHER 1977) while much shorter SCs characterize the chiasmatic microchromosomes of the domestic fowl (SOLARI 1979; RAHN and SOLARI 1986) and the chromosomes of *Coprinus cinereus* (HOLM *et al.* 1981).

The distribution of RNs along SCs in *M. e. ehrenbergii* is also potentially informative from the standpoint of the regulation of meiotic exchange. The RNs are neither localized to one or a few specific locations within bivalents nor are they evenly distributed along the entire SCs. Instead RNs are found in all positions along SCs but with markedly higher frequencies centrally decreasing in frequency particularly towards the attached telomeric ends. This distribution is not inherently surprising since RN and chiasma distributions of this form are commonly observed in entire, fully paired, bivalents (*e.g.*, CARPENTER 1979b; BERNELOT-MOENS and MOENS 1986; HENDERSON 1963; LAURIE and JONES 1981), but it is nevertheless interesting that the same characteristic is shown by short SC stretches representing probably less than 10% of the lengths of the bivalents concerned. These observations therefore emphasize that although *M. e. ehrenbergii* spermatocytes show striking chiasma localization at the light microscope level, this does not translate into fixed sites of exchange at the fine structure level.

This work was supported by a research grant (GR/C/98368) from the Science and Engineering Research Council.

LITERATURE CITED

ALBINI, S. M., and G. H. JONES, 1987 Synaptonemal complex spreading in *Allium cepa* and *A. fistulosum*. I. The initiation and sequence of pairing. Chromosoma **95:** 324–338.

ALBINI, S. M., and G. H. JONES, 1988 Synaptonemal complex spreading in *Allium cepa* and *A. fistulosum*. II. Pachytene observations. The SC karyotype and the correspondence of late recombination nodules and chiasmata. Genome **30:** 399–410.

BERNELOT-MOENS, C., and P. B. MOENS, 1986 Recombination nodules and chiasma localization in two Orthoptera. Chromosoma **93:** 220–226.

CARPENTER, A. T. C., 1975 Electron microscopy of meiosis in *Drosophila melanogaster* females. II. The recombination nodule—a recombination-associated structure at pachytene? Proc. Natl. Acad. Sci. U. S. A. **72:** 3186–3189.

CARPENTER, A. T. C., 1979a Synaptonemal complex and recombination nodules in wild-type *Drosophila melanogaster* females. Genetics **92:** 511–541.

CARPENTER, A. T. C., 1979b Recombination nodules and synaptonemal complex in recombination-defective females of *Drosophila melanogaster*. Chromosoma **75:** 259–292.

CARPENTER, A. T. C., 1987 Gene conversion, recombination nodules and the initiation of meiotic synapsis. Bioessays **6:** 232–236.

FLETCHER, H. L., 1977 Localised chiasmata due to partial pairing: a 3D reconstruction of synaptonemal complexes in male Stethophyma grossum. Chromosoma **65:** 247–269.

GILLIES, C. B., 1983 Ultrastructural studies of the association of homologous and non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Maydica **28:** 265–287.

GOLTENBOTH, F., 1973 DNA replication and chromosome structure of *Mesostoma* (*Turbellaria*). Chromosoma **44:** 147–181.

HENDERSON, S. A., 1963 Chiasma distribution at diplotene in the locust. Heredity **18:** 173–190.

HOLM, P. B., S. W. RASMUSSEN, D. ZICKLER, B. C. LU, and J. SAGE, 1981 Chromosome pairing, recombination nodules and chiasma formation in the Basidiomycete *Coprinus cinereus*. Carlsberg Res. Commun. **46:** 305–346.

HUSTED, L., and T. K. RUEBUSH, 1940 Comparative study of *Mesostoma ehrenbergii ehrenbergii* and *Mesostoma ehrenbergii wardii*. J. Morphol. **67:** 387–410.

JENKINS, G., and M. J. SCANLON, 1987 Chromosome pairing in a *Lolium temulentum* × *Lolium perenne* diploid hybrid with a low chiasma frequency. Theor. Appl. Genet. **73:** 516–522.

JONES, G. H., 1984 The control of chiasmata distribution. pp. 293–320. In: *Controlling Events in Meiosis, S. E. B. Symposium XXXVIII*, Edited by C. W. Evans and H. G. Dickinson. Company of Biologists, Cambridge.

KEYL, H. G., and F. GOLTENBOTH, 1972 Problems of the chromosome structure of *Mesostoma*. Chromosoma **36:** 292–304.

LAURIE, D. A., and G. H. JONES, 1981 Interindividual variation in chiasma distribution in *Chorthippus brunneus* (Orthoptera: Acrididae). Heredity **47:** 409–416.

OAKLEY, H. A., 1982 Meiosis in *Mesostoma ehrenbergii ehrenbergii* (*Turbellaria, Rhabdocoela*). II. Synaptonemal complexes, chromosome pairing and disjunction in achiasmate oogenesis. Chromosoma **87:** 133–147.

OAKLEY, H. A., 1985 Meiosis in *Mesostoma ehrenbergii ehrenbergii* (*Turbellaria, Rhabdocoela*). III. Univalent chromosome segregation during the first meiotic division in spermatocytes. Chromosoma **91:** 95–100.

OAKLEY, H. A., and G. H. JONES, 1982 Meiosis in *Mesostoma ehrenbergii ehrenbergii* (*Turbellaria, Rhabdocoela*). I. Chromosome pairing, synaptonemal complexes and chiasma localisation in spermatocytes. Chromosoma **85:** 311–322.

RAHN, M. I., and A. J. SOLARI, 1986 Recombination nodules in the oocytes of the chicken, *Gallus domesticus*. Cytogenet. Cell Genet. **43:** 187–193.

RASMUSSEN, S. W., and P. B. HOLM, 1978 Human meiosis. II. Chromosome pairing and recombination nodules in human spermatocytes. Carlsberg Res. Commun. **43:** 275–327.

SCHNEIDER, A., 1873 Untersuchungen uber Platyhelminthen. Jahres Oberhess Ges. Natur. Heilkunde **14:** 69–140.

SOLARI, A. J., 1977 Ultrastructure of the synaptic autosomes and the ZW bivalent in chicken oocytes. Chromosoma **64:** 155–165.

STACK, S., and L. ANDERSON, 1986 Two-dimensional spreads of synaptonemal complexes from solanaceous plants. III. Recombination nodules and crossing-over in *Lycopersicon esculentum* (tomato). Chromosoma **94:** 253–258.

VALKANOV, A., 1938 Cytologische Untersuchungen uber den Rhabdocoelen. Jahrb. Univ. Sofia Phys-Math. Fak. **34:** 321–402.

VON VOSS, H., 1914 Cytologische Studien an *M. ehrenbergii*. Arch. Zellforsch. **12:** 159–194.

VON WETTSTEIN, D., S. W. RASMUSSEN and P. B. HOLM, 1984 The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18:** 331–413.

WELLS, B., 1974 A convenient technique for the collection of ultra-thin serial sections. Micron **5:** 79–81

Communicating editor: A. T. C. CARPENTER