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Meiotic Structures in the Animal-Parasitic Nematode *Ascaris megalocephala:* Synaptonemal Complexes, Recombination Nodules, and Centrioles[1]

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Abstract: Two synaptonemal complexes (SCs) were present in the pachytene nuclei of *Ascaris megalocephala.* The SC was tripartite and comprised of two lateral elements (25 nm) with a striated central element (25 nm) and a central region of 65 nm. Spherical recombination nodules were observed to be associated only with the central element, although they are non-existent in the related *A. lumbricoides* var. *suum* (Goldstein, 1977). The SCs were attached to the nuclear envelope at only one end, while the other end was free in the nucleoplasm. This lack of bouquet formation of the chromosomes is consistent with all other nematodes studied. Morphologically distinct sex chromosomes were not observed, which differs from the presence of five Y-chromosomes present in *A. lumbricoides* var. *suum* (Goldstein and Moens, 1976). Centrioles (0.2 μm wide) reproduced by budding off the parental centriole. The centrioles consisted of nine singlet microtubules connected by an electron-dense proteinaceous ring. This structure is consistent with centrioles described in other nematodes, yet distinctly different from the centriole structure observed in most organisms in which it consists of nine triplet microtubules without any connecting ring. Multiple synaptonemal complexes, or polycomplexes, are found in *A. megalocephala* and *A. lumbriocoides* var. *suum.* They appear as stacked SC and are present inside the nucleus during zygotene and in the cytoplasm at pachytene.

Key words: Ascaris megalocephala, centriole, nematode, polycomplex, recombination nodule, synaptonemal complex.

Ascaris megalocephala, a parasitic nematode of horses, has a haploid chromosome number of n = 2. It was Boveri (1887) who showed that the two chromosomes present in meiosis appeared as many minute chromosomes during mitosis in the early embryo. This led to the discovery of "chromosome diminution," whereby somatic cells of the early embryonic cleavages lose portions of the chromosomes while germline chromosomes remain intact. The process has also been described in *A. lumbricoides* var. *suum* (Goldstein, 1978).

The ancestral species, *A. megalocephala,* (n = 2) gave rise to a number of different species via polyploidization, which is considered to be the mechanism for the origin of *A. lumbricoides* var. *suum* (n = 12). In such cases, there is often a change in the reproductive mode of the two species. However, both of these species reproduce via amphimixis. The synaptonemal complexes (SCs) structures found only in the nucleus in meiotic prophase I are identical. The morphology of the sex chromosomes may change with speciation. This is taken to the extreme in these two species whereby *A. megalocephala* has no visible sex chromosomes, yet *A. lumbricoides* var. *suum* has five Y-chromosomes (Goldstein, 1978; Goldstein and Moens, 1976). Other processes that are integral to meiosis also may change with speciation. Although crossing-over (recombination) occurs in all nematodes that reproduce via amphimixis, the mechanism may vary. For example, the "recombination nodule," a chromosomal structure observed at the ultrastructural level in meiotic prophase I nuclei, and which may be the equivalent of chiasmata, is absent in some nematodes of the same genus.

The components of meiosis in *A. megalocephala* have not been fully described or compared with *A. lumbricoides* var. *suum.* In this paper, we discuss the SCs, recombination nodules (RNs), and centrioles that are the integral components.

Materials and Methods

Ascaris megalocephala were collected from horses at the abattoir and immediately fixed in 4% glutaraldehyde, 0.05 M phosphate buffer, pH 6.3. The testes were removed, cut into 5-mm portions in consecutive series (beginning from the distal germinal end), and placed into fresh fixative for 2 hours. Post-fixation was in Dalton's osmium-chromic acid for 2 hours at room temperature, followed by dehydration through an alcohol and propylene oxide series, embedded in Epon, and stained with uranyl acetate and lead citrate (Fiske, 1966). Ultrathin sections were cut on an LKB Nova (Sweden) and examined on a Zeiss EM10 (Germany).

Computer analysis: Karyotypes of three pachytene nuclei were determined using reconstruction of entire nuclei from electron microscopy of serial ultrathin sections (Table 1). Two different computer packages were used for determination and three-dimensional analysis of the reconstructed nuclei: (i) METAMORPHOSIS for data entry from electron micrographs via a Houston Instruments Hi-Pad digitizer (Voight and Goldstein, 1987), and (ii) BIOGRAF 3-D for rotational and spatial analysis of interrelationships between chromosomes and associated nuclear structures (Peeples and Goldstein, 1989). The numbering system for SCs was based on relative length. The nuclear volumes were calculated using a subroutine in METAMORPHOSIS.

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TABLE 1. Synaptonemal complexes of *Ascaris megalocephala.*

	Nucleus 1		Nucleus 2		Nucleus 3		Average	
SC#	length	%	length	%	length	%	length	%
1	29.3	59.8	27.2	57.5	29.9	62.7	28.8	60.0
2	19.7	40.2	20.1	42.5	17.8	37.3	19.2	40.0
Total length (μm)		49.0		47.3		47.7		48.0
Nuclear volume (μm^3)		73.2		77.3		70.1		73.5

RESULTS

As in all other nematodes, the spermatocytes in the pachytene stage of meiosis prophase I were arranged peripherally around a central rachis in *A. megalocephala* (Favard, 1961). In the pachytene nuclei, only one end of the SC was attached to the nuclear envelope while the other end was free in the nucleoplasm (Fig. 1). This lack of bouquet formation of the chromosomes is consistent with all other nematodes studied. The SC appeared as a tripartite structure consisting of two lateral elements (each an average of 25 nm wide based on 50 measurements), one central element (an average of 25 nm based on 50 measurements), and the central region (an average of 65 nm based on 50 measurements). The central element was striated while the lateral elements were amorphous (Fig. 2). The overall width of the SC was an average of 115 nm (based on 50 measurements).

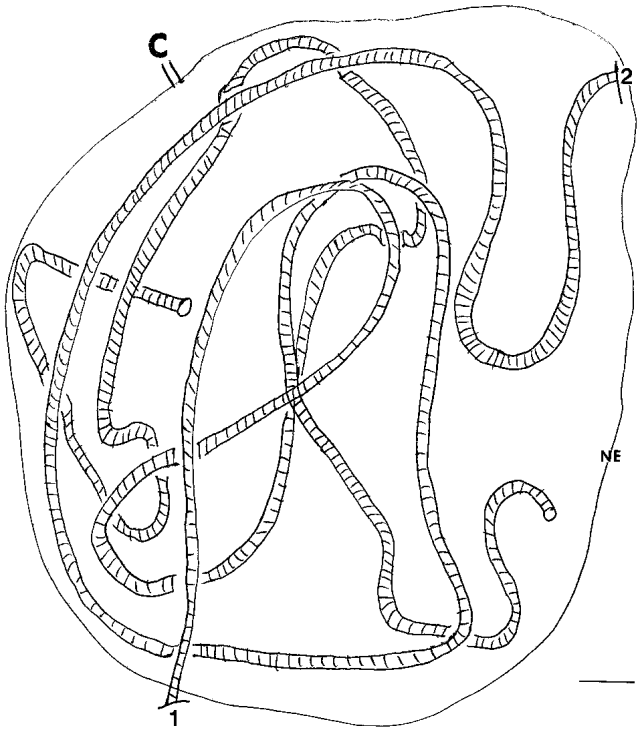

FIG. 1. Spermatocyte pachytene nucleus from *Ascaris megalocephala* reconstructed from 57 consecutive serial sections. The two synaptonemal complexes (SCs) are attached at one end to the nuclear envelope, and the opposite end (circle) is free in the nucleoplasm. The SCs are numbered according to length. The centriole (C) is associated with the nuclear envelope (NE). Bar equals 0.5 μm.

The length of the SCs ranged from 29.9 μm to 17.8 μm (Table 1). The total karyotype length was, on the average, 48 μm. The average nuclear volume was 73.5 μm^3. The structure of the SCs in *A. megalocephala* were identical to those reported in *A. lumbricoides* var. *suum* (Goldstein and Moens, 1976). Condensed heterochromatin was never observed to be associated with the SC in the specimens examined in this study. There were no recognizable sex chromosomes, which is different than the five Y-chromosomes present in *A. lumbricoides* var. *suum* (Goldstein and Moens, 1976).

Recombination nodules, which are the site of crossing-over between the homologous chromosomes, were associated with the central element of the SC in *A. megalocephala* (Fig. 2A). Only spherical RNs were observed, and not the ellipsoid type. Spherical RNs have been reported at zygotene and may facilitate homologous chromosome pairing (Ashley, 1994). This is different than in *A. lumbricoides* var. *suum,* where RNs were never observed (Goldstein, 1977, 1978; Goldstein and Moens, 1976).

Centrioles were present in all stages of meiotic prophase I in *A. megalocephala* and *A. lumbricoides* var. *suum* (Fig. 3). They were either associated with the nuclear envelope or in the cytoplasm. Reproduction appeared as budding off the parental centriole. The centrioles

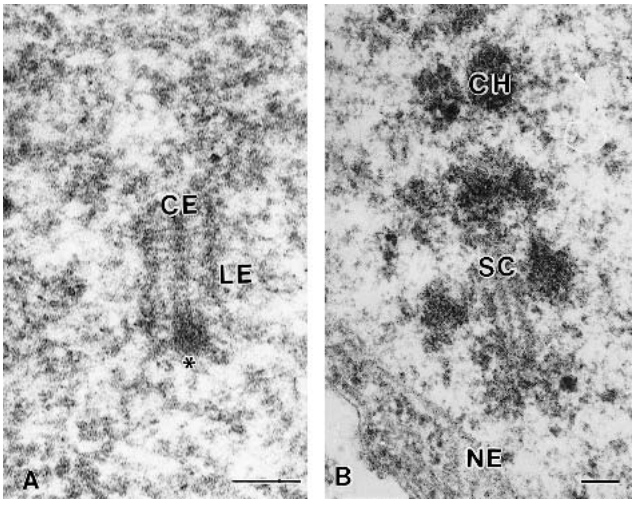

FIG. 2. The SCs in *Ascaris megalocephala* (A) and *A. lumbricoides* var. *suum* (B). Lateral elements (LE). Central element (CE). A recombination nodule (asterisk) is present in *A. megalocephala* but absent in *A. lumbricoides* var. *suum.* Chromatin (CH) Nuclear envelope (NE). Bar equals 0.1 μm in A and B.

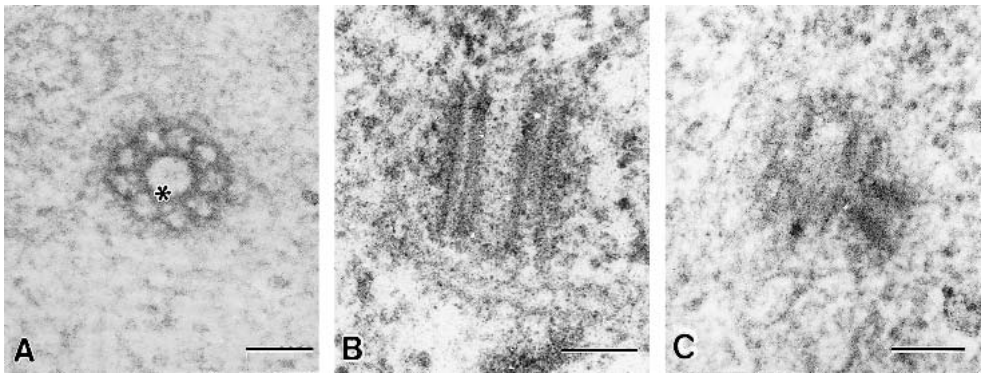

FIG. 3. Centrioles in *Ascaris megalocephala* and *A. lumbricoides* var. *suum.* Nine singlet microtubules (Fig. 2A, asterisk) connected by an electron-dense ring. Longitudinal view of the centriole (Fig. 2B). Replication of the centriole in both species is by budding off the parental centriole (Fig. 2C). The diam. of the centriole is 0.2 μm. Bar equals 0.1 μm in A-C.

consisted of nine singlet microtubules connected by an electron-dense proteinaceous ring. Such structure is consistent with centrioles described in other nematodes, yet distinctly different than the centriolar structure observed in all other organisms, which consists of nine triplet microtubules without any connecting ring. The average diameter of the centriole was 0.2 μm (based on 10 measurements).

In *A. megalocephala,* multiple SCs (polycomplexes) were observed inside the nucleus (associated with the nucleolus) and outside the nucleus (associated with the nuclear envelope) (Fig. 4). They consisted of short regions of SCs stacked together and were completely devoid of any associated chromatin. These polycomplexes were identical to those observed in *A. lumbricoides* var. *suum* (Fig. 4) (Bogdanov, 1977; Fill et al., 1977). Recombination nodules were not observed in the polycomplexes, which is consistent with the lack of chromatin in these structures.

DISCUSSION

Synaptonemal complexes: Ascaris megalocephala (n = 2), the ancestral form, and *A. lumbricoides* var *suum* (n = 12) had SC that were identical in structure. Because *A. lumbricoides* var. *suum* arose via polyploidization, which is a common mechanism in nematodes (Triantaphyllou, 1983), the SCs can be altered because polyploidization may be accompanied by either chromosome fragmentation or chromosome duplication. Polyploidization is

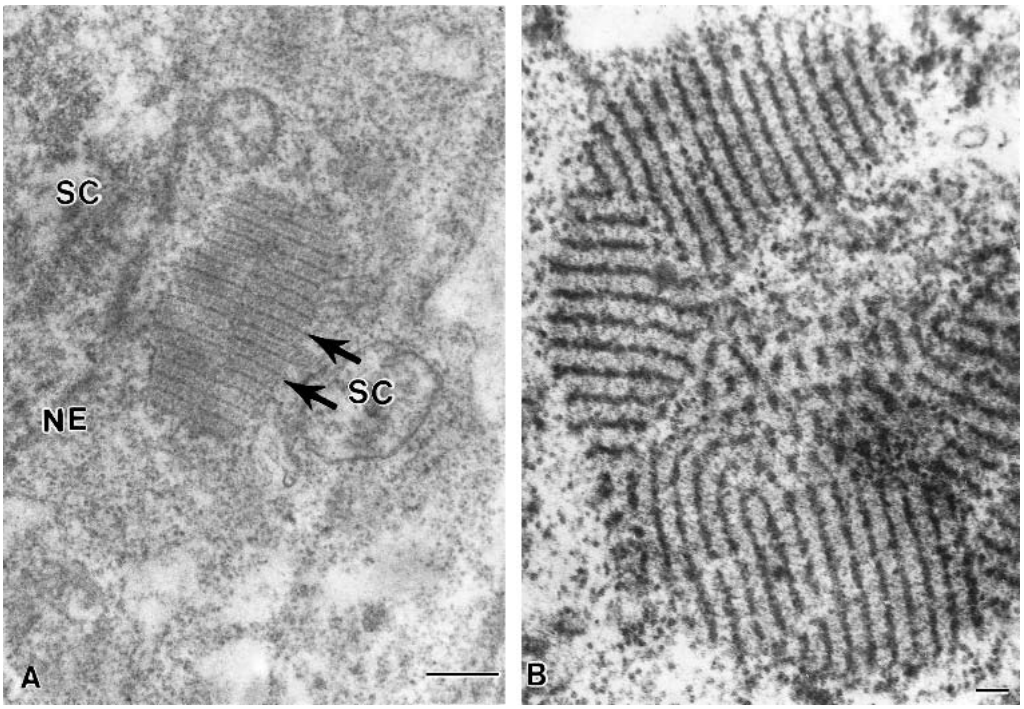

FIG. 4. Multiple SCs, or polycomplexes, are found in *Ascaris megalocephala* (A) and *A. lumbriocoides* var. *suum* (B). They appear as stacked SCs and are present inside the nucleus during zygotene and in the cytoplasm at pachytene. The polycomplexes shown in A and B are in the cytoplasm. Nuclear envelope (NE). Bar equals 0.5 μm in A and 0.1 μm in B.

also the mechanism for the evolution of a strain of *A. suum* with n = 24 (Mutafova, 1975), and the SCs are unaltered. This mechanism has been implicated in the evolution of chromosomes in other Ascaridida, for example, *Ascaridia galli* and *Ascaridia dissimilis* (2n = 10) (Mutafova, 1976), *Toxocara canis* (2n = 36), *Heterakis gallinarum* (2n = 10), and *Toxocara mystax* (2n = 20) (Triantaphyllou, 1983).

With polyploidization devoid of chromosome fragmentation, pairing of homologues could be normal, and the SC could be unchanged, as was described in the plant-parasitic nematode *Meloidogyne hapla* tetraploid (n = 34) (Goldstein and Triantaphyllou, 1978). When homologues pair normally, crossing-over and segregation of the chromosomes at anaphase are highly regulated to ensure genetic balance. In other organisms, there is an alternative way for pairing in polyploid chromosomes, called "switching of pairing partners." This can occur when three or four homologues each undergo a cross-over event with any of the others, which results in the unequal distribution of chromosomes to each of the gametes, decreased fertility, and fecundity. However, switching of partners has never been described in any nematode; thus, pairing of homologues occurs only via the normal pairing mechanisms.

Centrioles: In work using *A. megalocephala* (Boveri, 1887) the terms "centrosome" and "centriole" were first used. A centriole appears as a short cylinder, which is on average 0.15 µm in diam. and 0.5 µm long. Centrioles are generally paired, and at right angles to each other. The structure is conserved through evolution, such that organisms at all levels of the evolutionary scale have centrioles with similar structure: nine triplet microtubules arranged in a circle (Marshall and Rosenbaum, 1999). They are composed of a large number of polypeptides, including $\alpha/\beta/\delta/\epsilon$ tubulin (Dutcher, 2001) and centrin (Salisbury, 1995), as well as tektin filaments and their associated structural proteins (Steffen et al., 1994). At the ultrastructural level, the centrosomes are comprised of a pair of centrioles associated with pericentriolar material and tubulin. Centrioles are required to organize the centrosome in which they are embedded and are also needed for cilia and flagella (Hinchcliffe and Sluder, 2001).

Replication of centrioles is via budding. A procentriole (Fig. 3) appears on the parental centriole as a short cylinder that progressively elongates to reach to final size (Bobinnec et al., 1998). Before the cell enters meiosis, the single interphase centriole has replicated. The centrioles move to the poles, the nuclear envelope breaks down, and the microtubules, organized by that centriole in the centrosome, provide part of the mechanism for chromosome segregation. Failure of the cell to precisely control this duplication can result in aneuploidy, polyploidy, and other segregational abnormalities.

Centrioles found in nematodes are comprised of nine singlet microtubules, which are connected by a dense proteinaceous ring (Justine and Jamieson, 1999; Justine, 2002). This may be the result of loss of interactions between microtubular triplets or by the destabilization of the triplets themselves (Bobinnec et al., 1998). The structure is conserved throughout the Nematoda with the following as examples: *Heterodera betulae, H. glycines, Meloidogyne hapla, M. natalie, M. spartinae, M. carolinensis, Ascaris lumbricoides* var *suum, A. megalocephala* (Favard, 1961), *Caenorhabditis elegans* (Wolf et al., 1978), *Sphaerolaimus hirsutus* (Noury-Srairi et al., 1993), *Trichinella spiralis* (Slomianny et al., 1981), *Heterakis gallinarum* (Lee, 1971), *Dipetalonema viteae* (McLaren, 1973), *Capillaria muris* (Wright and Sommerville, 1985), and *Nipponstrongylus brasiliensis* (Jamuar, 1966). Variations have been reported. For example, in the nematode *Heligmosomoides polygyrus,* the centriole is comprised of 10 singlet microtubules connected by a dense ring (Mansir and Justine, 1995), while in *Gastromermis* sp., it is comprised of nine doublets (Poinar and Hess-Poinar, 1993).

Recombination nodules: Recombination nodules are electron-dense structures located in the central region, or attached to the lateral elements, of SCs (Fig. 2). They are approximately 100 nm in diam. and were first discovered in *Drosophila* (Carpenter, 1975). Recombination nodules are observed during zygotene and pachytene of Prophase I of meiosis, at which time crossing-over has occurred. There may be a direct correlation between the presence of RNs and the location of chiasmata in most organisms (Ashley, 1994). In nematodes, there is no correlation between the length of the SCs and the occurrence of RNs (Goldstein and Triantaphyllou, 1995).

In mammals, recombination nodules exist at two separate times during Prophase I. "Early" RNs form at zygotene and facilitate homologous chromosome pairing along with the formation of the SC. "Late" RNs mediate reciprocal recombination resulting in crossing-over and chiasmata formation. These two types are morphologically distinct, such that the early RNs are spherical nodules and the late RNs are bar-shaped (Ashley, 1994). In the present study involving only nematodes, only spherical RNs were observed; however, their exact role has yet to be determined.

Spherical RNs have been identified in *Meloidoigyne hapla* (Goldstein and Triantaphyllou, 1978) and *M. spartinae* (Goldstein and Triantaphyllou, 1995). One study has shown putative RNs in *A. suum* (Bogdanov, 1977); however, this was not seen by Goldstein (1977). This may be a real difference between subspecies. Recombination nodules were not observed in the amphi-

mictic nematodes *M. microtyla, M. carolinensis,* and *M. megatyla.*

Literature Cited

Ashley, T. 1994. Mammalian meiotic recombination: A reexamination. Human Genetics 94:587–593.

Bobinnec, Y., A. Khodjakov, L. Mir, C. Rieder, B. Edde, and M. Bornens. 1998. Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. Journal of Cell Biology 143:1575–1589.

Bogdanov, Y. 1977. Formation of cytoplasmic synaptonemal-like polycomplexes at leptotene and normal synaptonemal complexes at zygotene in *Ascaris suum* male meiosis. Chromosoma 61:1–21.

Boveri, T. 1887. Uber differenzierung der zellkerne wahrend der furchung des eis von *Ascaris megacephala.* Anatomischer Anzeiger 2: 688–693.

Carpenter, A. T. C. 1975. Electron microscopy and recombination nodules in the wild-type *Drosophila melanogaster* females. Genetics 92: 511–541.

Dutcher, S. 2001. The tubulin fraternity: Alpha to eta. Current Opinions in Cell Biology 13:49–54.

Favard, P. 1961. Évolution des ultrastructures cellulaires au cours de la spermatogenèse de l'ascaris (*Ascaris megalocephala,* Schrank = Parascaris equorum, Goerze). Annales des Sciences Naturelles, Zoologie et Biologie Animale, 12° Série, 3:52–152.

Fiil, A., P. Goldstein, and P. B. Moens. 1977. Precocious formation of synaptonemal-like polycomplexes and their subsequent fate in female *Ascaris lumbricoides* var. *suum.* Chromosoma 65:21–35.

Fiske, S. 1966. An adaptation of Reynold's lead citrate stain for high resolution autoradiography. Journal de Microscopie 5:355–360.

Goldstein, P. 1977. Spermatogenesis and spermiogenesis in *Ascaris suum.* Journal of Morphology 154:317–338.

Goldstein, P. 1978. Ultrastructural analysis of sex determination in *Ascaris lumbricoides* var. *suum.* Chromosoma 66:59–69.

Goldstein, P., and P. B. Moens. 1976. Karyotype analysis of *Ascaris lumbricoides* var. *suum.* Chromosoma 58:101–111.

Goldstein, P., and A. Triantaphyllou. 1978. Occurrence of synaptonemal complexes and recombination nodules in a meiotic race of *Meloidogyne hapla* and their absence in a mitotic race. Chromosoma 68:91–100.

Goldstein, P., and A. Triantaphyllou. 1995. Pachytene karyotype analysis of the nematode *Meloidogyne spartinae* in relation to the genus *Meloidogyne.* Cytobios 83:91–103.

Hinchcliffe, E., and G. Sluder. 2001. It takes two to tango: Understanding how centrosome duplication is regulated throughout the cell cycle. Genes and Development 15:1167–1181.

Jamuar, M. P. 1966. Studies of spermiogenesis in a nematode, *Nippostrongylus brasiliensis.* Journal of Cell Biology 31:381–396.

Justine, J-L. 2002. Male and female gametes and fertilization. Pp 73–119 *in* D. Dee, ed. The biology of nematodes. London & New York: Taylor & Francis.

Justine, J-L., and B. Jamieson. 1999. Nematoda. Pp 183–266 *in* B. Jamieson, ed. Progress in male gamete ultrastructure and phylogeny. New Delhi & Calcutta: Oxford & IBH.

Lee, D. L. 1971. The structure and development of the spermatozoon of *Heterakis gallinarum* (Nematoda). Journal of Zoology 164: 181–187.

Mansir, A., and J-L. Justine. 1995. Centrioles with ten singlets in spermatozoa of the parasitic nematode *Heligmosomoides polygyrus.* Museum National d'Histoire Naturelle, Paris 166:119–128.

Marshall, W., and J. Rosenbaum. 1999. Cell division: The renaissance of the centriole. Current Biology 9:R218–R220.

McLaren, D. J. 1973. The structure and development of the spermatozoon of *Dipetalonema viteae* (Nematoda: Filarioidea). Parasitology 66:447–463.

Mutafova, T. 1975. Morphology and behavior of sex chromosomes during meiosis in *Ascaris suum.* Zeitschrift fur Parasitenkunde 46:291–295.

Mutafova, T. 1976. Comparative cytological studies of mitotic and male meiotic karyotype of *Ascaridia dissimilis* and *Ascaridia galli.* Zeitschrift fur Parasitenkunde 48:239–245.

Noury-Sraïri, N., N. Gourbault, and J. L. Justine. 1993. The development and evolution of actin-containing organelles during spermiogenesis of a primitive nematode. Biology of the Cell 79:231–241.

Peeples, W., and P. Goldstein. 1989. BioGraf 3-D: An algorithm for optimization and graphical representation of nuclear structure spatial distributions. Cytobios 58:109–1123.

Poinar, G. O. J., and R. T. Hess-Poinar. 1993. The fine structure of *Gastromermis* sp. (Nematoda: Mermithidae) sperm. Journal of Submicroscopic Cytology and Pathology, 25, 417–431.

Salisbury, J. 1995. Centrin, centrosomes, and mitotic spindle poles. Current Opinions in Cell Biology 7:39–45.

Slomianny, C., C. Herbaut, A. Vernes, and J. Biguet. 1981. An ultrastructural study of spermatogenesis in *Trichinella spiralis* Owen 1835. Zeitschrift fur Parasitenkunde 64:207–215.

Steffen, W., E. Fajer, and R. Linck. 1994. Centrosomal components immunologically related to tektins from ciliary and flagellar microtubules. Journal of Cell Science 107:2095–2105.

Triantaphyllou, A. 1983. Chromosomes in evolution of nematodes. Pp 77–101 *in* A. K. Sharma and A. Sharma, eds. Chromosomes in evolution of eukaryotic groups, vol. II. Boca Raton, FL: CRC Press.

Voight, M., and P. Goldstein. 1987. Metamorphosis: Computer-assisted rotational analysis and interspatial distribution of nuclear structures from serial ultrathin sections. Cytobios 51:193–207.

Wolf, N., D. Hirsh, and J. R. McIntosh. 1978. Spermatogenesis in males of the free-living nematode, *Caenorhabditis elegans.* Journal of Ultrastructure Research 63:155–169.

Wright, E. J., and R. Sommerville. 1985. Spermatogenesis in a nematode *Nippostrongylus brasiliensis.* International Journal for Parasitology 15:283–299.