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Three-Dimensional Ultrastructural Karyotype Analysis from the Meiotic Parthenogenetic Nematode *Heterodera betulae*[1]

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Abstract: Meiotic chromosome structure and function are described in the plant-parasitic nematode *Heterodera betulae.* Twelve synaptonemal complexes (SCs) were reconstructed from pachytene nuclei; therefore, n=12 is predicted for this species. Morphologically distinct sex chromosomes were not observed. Only one end of the SC is attached to the nuclear envelope, and there is no bouquet arrangement at pachytene. The structure of the SC in this meiotic parthenogenetic nematode was different than in other nematodes that reproduce via amphimixis; a striated central element with transverse filaments was not observed. Multiple SCs, or polycomplexes, were present in the nucleus. Recombination nodules were not observed. The centrioles were comprised of nine doublet microtubules connected by a ring, which is a distinct modification from the typical nine triplet microtubules without any interconnecting structure.

Key words: chromosome, cyst nematode, cytogenetics, *Heterodera betulae,* recombination nodules, synaptonemal complex.

A morphologically distinct new species of the genus *Heterodera* A. Schmidt, 1871 was first described in 1969 (Riggs et al., 1969). This new species is cytologically distinct from the rest of the known *Heterodera* species because it has a haploid chromosome number of n = 12 or 13 as compared to n = 9 (Triantaphyllou, 1970). Analysis of DNA content determined that the higher chromosome number of this species resulted from the addition of chromosomes rather than fragmentation of the chromosomes of the original karyotype (Lapp and Triantaphyllou, 1972). Thus, *H. betulae* may have arisen via duplication of chromosomes (Triantaphyllou, 1983).

Cytological analysis revealed that maturation of oocytes of *H. betulae* is by typical meiosis and reproduction is by parthenogenesis (Triantaphyllou, 1970, 1979). This "meiotic parthenogenesis" is characterized by the maturation of oocytes following the conventional meiotic cycle and involves pairing of homologous chromosomes and formation of bivalents. Two maturation divisions that follow lead to the formation of two polar nuclei and an egg pronucleus with the haploid chromosomal complement. The diploid chromosome number is reestablished usually by fusion of the egg pronucleus with the second polar nucleus (Triantaphyllou, 1983). Synapsis of homologous chromosomes occurs during the zygotene stage of meiosis prophase I. The haploid chromosome number is n = 12 (2n = 24) in 95% of the female nematodes studied and n = 13 in the remaining 5%.

The current study was conducted to establish the number of chromosomes in this species via three-dimensional reconstruction of ultrathin sections and to compare the data from other nematodes. Only *H. glycines* from this genus has been previously examined. The phylogenetic relationship of the Heteroderea is not clear; thus, this may help elucidate the relationships among these species (Triantaphyllou, 1970; Triantaphyllou and Hirschmann, 1980).

Materials and Methods

Heterodera betulae was propogated on river birch seedlings, *Betula nigra* L., in the greenhouse at 20 °C to 30 °C (Triantaphyllou, 1970). The life cycle was 52 days at the optimal temperature of 28 °C. Males were rarely encountered in populations, but their presence would allow cross-fertilization (Riggs et al., 1969). The restrictive temperature for reproduction was determined to be 31 °C or above, and development was very slow and below 20 °C. Successful population propagations from single second-stage juveniles showed that males were not necessary for reproduction (Riggs et al., 1969). Fourth-stage juveniles and young females obtained from infected roots were prepared for electron microscopy following Goldstein and Slaton (1982).

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.

Results

The general morphology of the telogonic gonad was similar to all other nematodes described, e.g. *Ascaris*

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This paper is dedicated to Professor Anastasios C. Triantaphyllou, a true "Renaissance Man," mentor, friend, and advisor. His enthusiasm for science and life continues through his students and *their* students. Thus, we say "Strength from Generation to Generation." We are honored to have been included in his family.

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TABLE 1. Synaptonemal complexes of *Heterodera betulae* (n = 12).

	Nucleus 1		Nucleus 2		Nucleus 3		Average	
SC#	length	%	length	%	length	%	length	%
1	5.0	2.0	4.2	1.5	6.1	2.4	5.1	2.0
2	5.9	2.4	4.5	1.7	6.7	2.7	5.7	2.2
3	6.8	2.7	8.3	3.1	7.5	3.0	7.5	2.9
4	7.5	3.0	8.7	3.2	7.7	3.1	8.0	3.1
5	13.1	5.2	8.9	3.3	10.1	4.0	10.7	4.1
6	18.7	7.5	17.3	6.4	18.1	7.2	18.0	7.0
7	19.0	7.6	21.2	7.8	18.7	7.4	19.6	7.6
8	28.1	11.2	27.8	10.2	21.0	8.3	25.6	9.9
9	28.9	11.5	33.6	12.4	27.2	10.8	29.9	11.6
10	29.9	11.9	41.0	15.1	34.6	13.7	35.3	13.7
11	35.7	14.2	47.3	17.4	40.7	16.1	41.2	16.0
12	52.0	20.8	48.6	17.9	53.7	21.3	51.4	19.9
Total length (μm)		250.6		271.4		252.1		258.0
Nuclear volume (μm^3)		158		162		160		160

suum (Goldstein, 1978), *Meloidogyne hapla* (Goldstein and Triantaphyllou, 1978a, 1978b), *Meloidogyne carolinensis* (Goldstein and Triantaphyllou, 1982), *Meloidogyne spartinae* (Goldstein and Triantaphyllou, 1996), *H. glycines* (Goldstein and Triantaphyllou, 1979), *Caenorhabditis elegans* (Goldstein and Slaton, 1982), and *Panagrolaimus davidi* (Goldstein and Wharton, 1996). Prior to the pachytene stage of meiosis prophase I, the oogonial cells were arranged in a honeycomb pattern with no definite organization. At pachytene, the honeycomb arrangement of the cells within the gonad changed into an organized pattern, such that the nuclei were arranged peripherally around a central rachis, like a wheel with spokes. The attachment of all cells to the central rachis at this stage accounted for their developmental synchrony.

Within the pachytene nuclei, 1 nucleolus was present and 12 SCs were observed. Thus, the haploid chromosome number is predicted to be n = 12. These SCs consisted of two lateral elements, each 20 nm wide, and a non-striated central element 60 nm wide Transverse filaments were not observed (Fig. 1a). The average length of the SCs ranged from 5.1 μm to 51.4 μm (Table 1). The total karyotype length averaged 258 μm, and the average nuclear volume was 160 μm^3. Centrioles were observed near the cell membrane (Fig. 1b). Centriolar structure was similar to that observed in other nematodes, such that the usual structure of nine triplet microtubules was reduced to nine doublet microtubules connected by a ring. A centrosome was present, which consisted of microtubules that emanated from the centriolar pair. Multiple SCs, or polycomplexes, were present in the pachytene nuclei (Fig. 1c).

The telomeric association of the SC in *H. betulae* and the nuclear envelope deserves special attention. In most organisms, both ends of the SC are attached to the nuclear envelope at pachytene. However, in nematodes, only one end is attached to the nuclear envelope, while the other end is free in the nucleoplasm. Unlike *H. glycines,* there was no heterochromatic knob at the

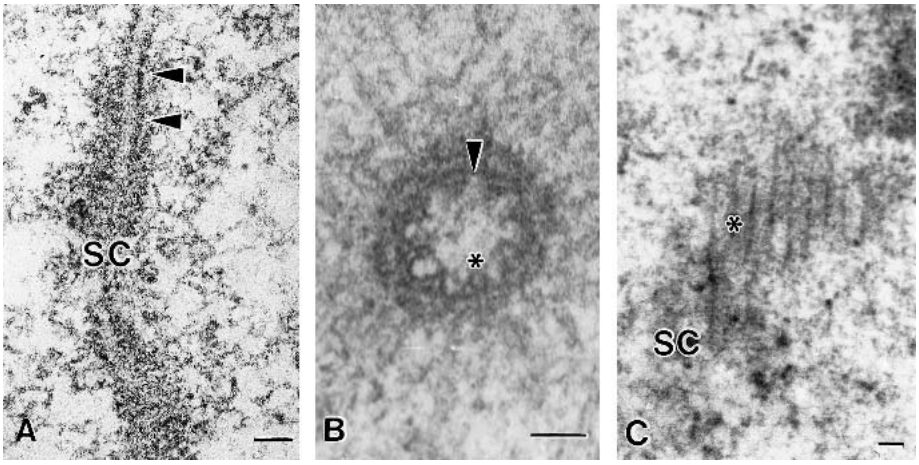

FIG. 1. a) Synaptonemal complex (SC) of the meiotic parthenogenetic *Heterodera betulae.* Cross-striations are not observed in the central region between the lateral elements (arrowheads). Bar equals 0.2 μm. b) Centriole in an *H. betulae* oocyte. Nine doublet mircotubules (asterisk) are connected by a dense, dark-staining ring (arrowhead). Bar equals 0.1 μm. c) Multiple SCs (polycomplexes) are present in pachytene nuclei of *H. betulae.* The polycomplex consists of stacked (asterisk), discarded segments of SCs. Bar equals 0.1 μm.

free end of the SC in *H. betulae;* thus, it was not possible to determine if the same end of the SC attached each time to the nuclear envelope. It is expected that both ends are competent to attach, as this was shown in *M. spartinae* (Goldstein and Triantaphyllou, 1996). In that case, the SC carrying the nucleolar organizing region was able to attach with either end to the nuclear envelope. Also, in older specimens of *C. elegans,* but not younger ones, both ends of the SC were competent to attach to the nuclear envelope (Goldstein and Curis, 1987). In *H. betulae,* there was no bouquet formation of attachment; thus, the ends were dispersed randomly throughout the nucleus instead of being restricted to a small region on the nuclear envelope (Fig. 2). In addition, there were no morphologically distinct sex chromosomes.

Discussion

Synaptonemal complexes are tripartite, proteinaceous structures that are present in the prophase 1 stage of meiosis (von Wettstein et al., 1984). The SC regulates the association between homologous chromosomes and maintains this association during chromosome pairing. The SC also coordinates recombination and proper segregation of the chromosomes. At the beginning of prophase 1, axial cores are recognizable in the homologous chromosomes. These axial cores become the lateral elements of the completed SC as meiosis prophase 1 progresses. The central region lies between the two lateral elements and consists of two distinctive substructures. The central element lies parallel to and equidistant between the two lateral elements, whereas the transverse filaments (TF) lie perpendicular to the long axis of the complex (Dong and Roeder, 2000). Some TFs appear to span the full width of the SC, bridging the space between the two lateral elements, while other TFs are shorter and connect a single lateral element to the central element (Schmekel and Daneholt, 1995). The structure of the SC is conserved throughout evolution, leading to an essentially uniform design for most organisms regardless of their position on the evolutionary ladder.

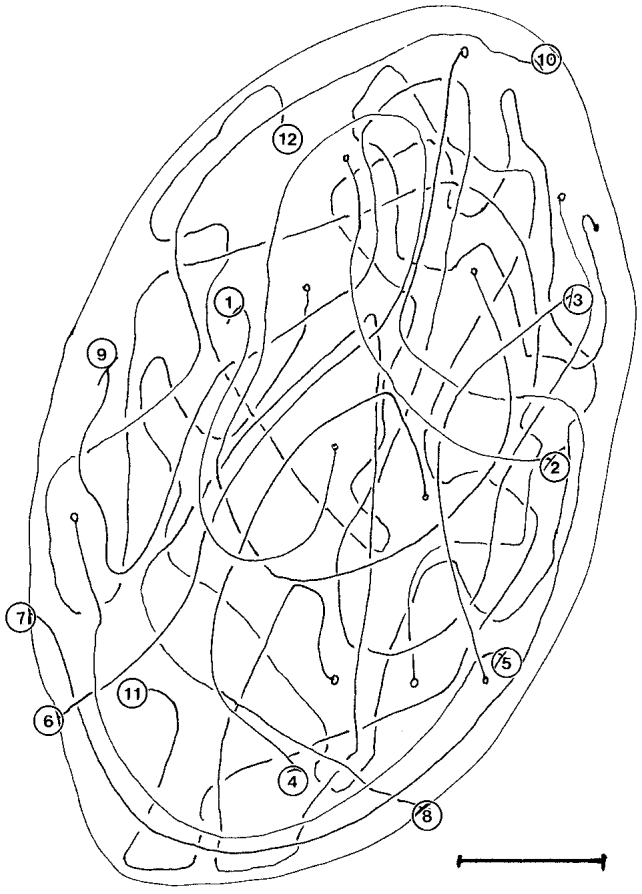

Fig. 2. Reconstruction of a pachytene nucleus from 69 serial sections in *H. betulae.* The SCs (numbered) are attached at only one end (telomere) to the nuclear envelope, while the other end is free in the nucleoplasm. There is no bouquet formation, i.e. no clustering, as evidenced by the distribution of the attachment sites.

The formation of the SC in *H. betulae* occurs without prior axial core formation. The lateral elements are apparently organized from a pool of precursors simultaneously with the SC (Schmekel et al., 1993). Transverse filaments were not observed in *H. betulae;* instead, the central element consisted of a linear non-striated structure. There is also a lack of morphologically identifiable transversing filaments in the central region of *M. hapla* (Goldstein and Triantaphyllou, 1978a, 1978b).

The SC is attached at only one telomeric end, while the other end is free in the nucleoplasm. The attached end may be randomly selected or there may be a selective mechanism that has not yet been delineated. Due to the single-end telomeric association, coiling of the SC is never observed; therefore, this might be a mechanism that serves to enhance segregation of the chromosomes (Zetka and Muller, 1996). In *A. suum,* the unattached telomeric end has been shown to be capable of acting as a centromere such that these regions have microtubules and kinetic activity associated with them (Zetka and Muller, 1996).

Recombination nodules are round or ellipsoid structures observed on the SC and represent the site of recombination. They are essentially the equivalent of the chiasma observed at the light microscope level. Although recombination nodules (RNs) have been reported in diploid and tetraploid *M. hapla* Race A, such structures were not observed in *M. megatyla, M. microtyla, M. carolinesis, H. glycines* (Goldstein and Triantaphyllou, 1979), or *P. davidi* (Goldstein and Wharton, 1996). This is also true for *H. betulae.* It is interesting that RNs are present in the *M. hapla* strain (which are meiotic parthenogenetic with aberrant central region morphology) because this is not the rule for the genus or other nematodes (Goldstein and Triantaphyllou, 1982). For example, there are no RNs observed in *Caenorhabditis elegans* (Goldstein and Slaton, 1982) or *Ascaris lumbricoides* var. *suum* (Goldstein and Moens,

1976). However, RNs are present in *A. megalocephala* (Bogdanov, 1977; P. Goldstein, unpub.).

Nematodes, e.g. *A. lumbricoides* var. *suum* (Goldstein, 1978) and *C. elegans* (Goldstein and Slaton, 1982), have distinct sex chromosomes. However, similar to *H. betulae,* most nematodes have no recognizable sex chromosomes. In some nematodes there may be regions along the SC that are modified, which may represent regions containing sex-associated genes. For example, the "modified SC regions" (MSC) in *H. glycines* are regions where the normal SC becomes disorganized as it enters the heterochromatic knob (Goldstein and Triantaphyllou, 1979). In other cases, where the SC is associated with heterochromatin (i.e., centromeric regions), the SC does not undergo a structural change (Gillies, 1973). Often, specific modifications along the SC suggest specific function, and this region has been theorized to be the location of sex-determining chromatin (Goldstein and Triantaphyllou, 1979).

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