Gametogenesis and chromosome complement in Cylindrocorpus longistoma and C. curzii

by

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Gametogenesis in C. longistoma and C. curzii follows the general pattern of most bisexual and hermaphroditic animals. Mitotic gonial divisions occur in the germinative zone of the gonads. Oocytes and spermatocytes undergo normal meiosis following a period of maturation. The diploid number is restored by fusion of the sperm and egg pronuclei. C. longistoma and C. curzii are distinct genetic species with different chromosomal mechanisms of sex determination. XX-XY and XX-XO sex determining mechanisms are inferred for C. longistoma and C. curzii, respectively.

Gametogenesis in free-living nematodes has been studied rather extensively in the Rhabditidae, and to a lesser extent in the Diplogasteridae. Thus far, no cytological information is available for any members of the Cylindrocorporidae. Cytogenetic studies can be instructive in the elucidation of taxonomic as well as phylogenetic relationships among free-living nematodes. This paper describes cytological studies to elucidate the gametogenesis and chromosome complement of Cylindrocorpus longistoma (Stefanski) and C. curzii (T. Goodey).

Materials and Methods

Single female populations of C. longistoma and C. curzii isolated from trickling filter effluent of the Urbana-Champaign Sanitary District Sewage Treatment Plant at Urbana, Illinois, were propagated monoxenically on Aerobacter aerogenes (Kruse) Beijerinck, growing on Chang's medium (Chang, 1958).

The staining technique used for studying chromatid behavior during gametogenesis was a combination of that described by Triantaphyllou & Hirschmann (1966) and Hechler (1968). Water suspensions containing adults, eggs and larval stages were fixed for 30 min in Carnoy fixative (absolute ethyl alcohol, six parts; chloroform, three parts; glacial acetic acid, one part). The nematodes were then transferred to 2% acetic-orcein for staining. After six to twelve hours at room temperature, during which time adequate staining was achieved, a drop of the nematode suspension in the stain was added to a drop of 45% glacial acetic acid on a microslide. A coverslip was applied and its edges sealed with Zut. The application of gentle pressure to the coverslip expelled the gonads from the body of the nematodes and thus aided subsequent examination of the chromosomes.
RESULTS

The various regions of the female reproductive system of *C. longistoma* and *C. curzii* are morphologically similar. The gonads reflex and cross each other near the vulva. The ovary occupies the distal dorsal limb of the gonad and is clearly divided into a short germinative zone and a rather extensive growth zone. A narrow anucleate constriction leads from the growth zone of the ovary to an oval-shaped spermatheca. The shape of the spermatheca frequently changes in gravid females. However, its location can be identified by the accumulation of large numbers of sperm. The spermatheca is followed by the uterus which is morphologically differentiated into two regions. The distal region consists of six large granular cells whose morphology depends on the physiological state of the gonad. The proximal portion consists of a single layer of thin-walled somatic cells. Two pyriform muscular structures, one on either side of the vulva, function as ovijectors.

Oogenesis

Oogenesis in *C. longistoma* and *C. curzii* follows the same pattern. Mitotic oogonial divisions begin in the germinative zone of the ovary of larvae during the third molt and continue for several days in the adult stage. Such divisions are unsuitable for karyotype analysis because the chromosomes remain indiscrete throughout all stages of the mitotic cycle. Although the prophase chromosomes (Fig. 1) stain very deeply, it is difficult to characterize individual chromosomes. A similar situation exists during metaphase when the chromosomes contract and move close to each other on a common metaphase plate (Fig. 2). Consequently, the chromosomes cannot be counted or their number estimated.

The oocytes increase in size as they migrate along the vegetative zone of the ovary, but their chromosomes gradually lose stainability and become invisible. Thus the intermediate stages of meiotic prophase I cannot be studied. The chromosomes regain their stainability in oocytes approaching the proximal end of the ovary. The metaphase stage of the first maturation division is most suitable for chromosome counts. During early metaphase, the chromosomes appear as short rods and the bivalents are clearly visible. Later they contract and move close to each other until each bivalent appears as a compact body. Six bivalent chromosomes were always counted for *C. longistoma* (Fig. 3) whereas seven bivalent chromosomes were observed in *C. curzii* (Fig. 4). The chromosomes of both species are of approximately the same size and are morphologically indistinguishable. One sperm penetrates each oocyte as the latter migrates through the spermatheca which usually contains several spermatozoa (Fig. 5). After the oocyte enters the uterus, the egg nucleus migrates to its periphery and soon advances to anaphase I. As the dyads move apart, the spindle of the egg nucleus turns through 90° so that at telophase I the first polar nucleus is parallel to the longitudinal axis and situated near the periphery of the oocyte (Fig. 6).

The second maturation division occurs while the oocyte is still within the