Chromosome Number and Reproduction in *Mononchoides changi* (Nematoda: Diplogasterinae)

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Abstract: Mononchoides changi Goodrich, Hechler, and Taylor reproduces by amphimixis. The female has 14 chromosomes, the male 13. Sperm cells have 6 or 7 chromosomes. During oogenesis three polar nuclei and the egg pronucleus are formed. Fusion of sperm and egg pronuclei was not observed, but in one egg at metaphase I, two chromosome groups, one larger than the other, were seen aligned side by side and were presumed to be from male and female pronuclei. Non-fertilized eggs are laid, but these disintegrate without cleaving. *Key Words:* Chromosome number, Reproduction, *Mononchoides changi.*

The reproductive mechanisms and chromosome numbers of nematodes in the superfamily Diplogasteroidea are known only for comparatively few species in the group, and the classification of some of these species is uncertain. Maupas (5) counted 12 chromosomes in sperm cells of *Diplogaster robustus* Maupas [made a *species inquirenda* by Goodey (1)] and he believed this species reproduced hermaphroditically. Mulvey (6) found 6 chromosomes in the sperm cells of an unidentified diplogasterid, as well as 6 chromosomes in both sister nuclei of a division he believed to be Anaphase I of oogenesis. He found sperm within the egg cell but did not determine whether reproduction was amphimictic or hermaphroditic. Hirschmann (4) found that *Mesodiplogaster lheritieri* (Maupas) reproduced bisexually, and both Potts (8) and Hirschmann (4) reported that *M. maupasi* (Potts) (syn. *Diplogaster biformis* Hirschmann) reproduced hermaphroditically. Triantaphyllou and Hirschmann (9) found sperm cells with both 5 and 6 chromosomes in *M. lheritieri.* Honda (3) showed that females of the amphimictic *Micoletzkya aerivora* (Cobb, in Merrill and Ford) produced non-fertile eggs in the absence of males, which disintegrated quickly without cleavage soon after they were laid. Pillai and Taylor (7) believed that both *Paroigolaimella bernensis* (Steiner) and *Fictor anchicoprophaga* (Paramonov) reproduced bisexually and had a haploid number of 6 chromosomes.

This is a report on the chromosome number and mode of reproduction in *Mononchoides changi* Goodrich, Hechler, and Taylor.

MATERIALS AND METHODS

Mononchoides changi was reared in petri dish culture on an amoeba *Naegleria gruberi* (Schardinger)/bacterium combination growing on sucrose-tryptose agar as reported by Goodrich, Hechler, and Taylor (2). Adult nematodes were killed and fixed 25 min or longer in Carnoy's solution. The fixed nematodes were transferred to a drop of acetic orcein on a slide, a coverslip was added, and the mount sealed with Zut. After staining 12 hr or longer the nematodes were examined and selected nuclei were photographed. Occasionally nuclei were flattened by gentle pressure on the coverslip to get more accurate chromosome counts. To establish monolarval cultures, nematodes in the pre-adult stage or younger were transferred individually to a drop of bacterial slime on ¼ strength sucrose-tryptose agar plates.

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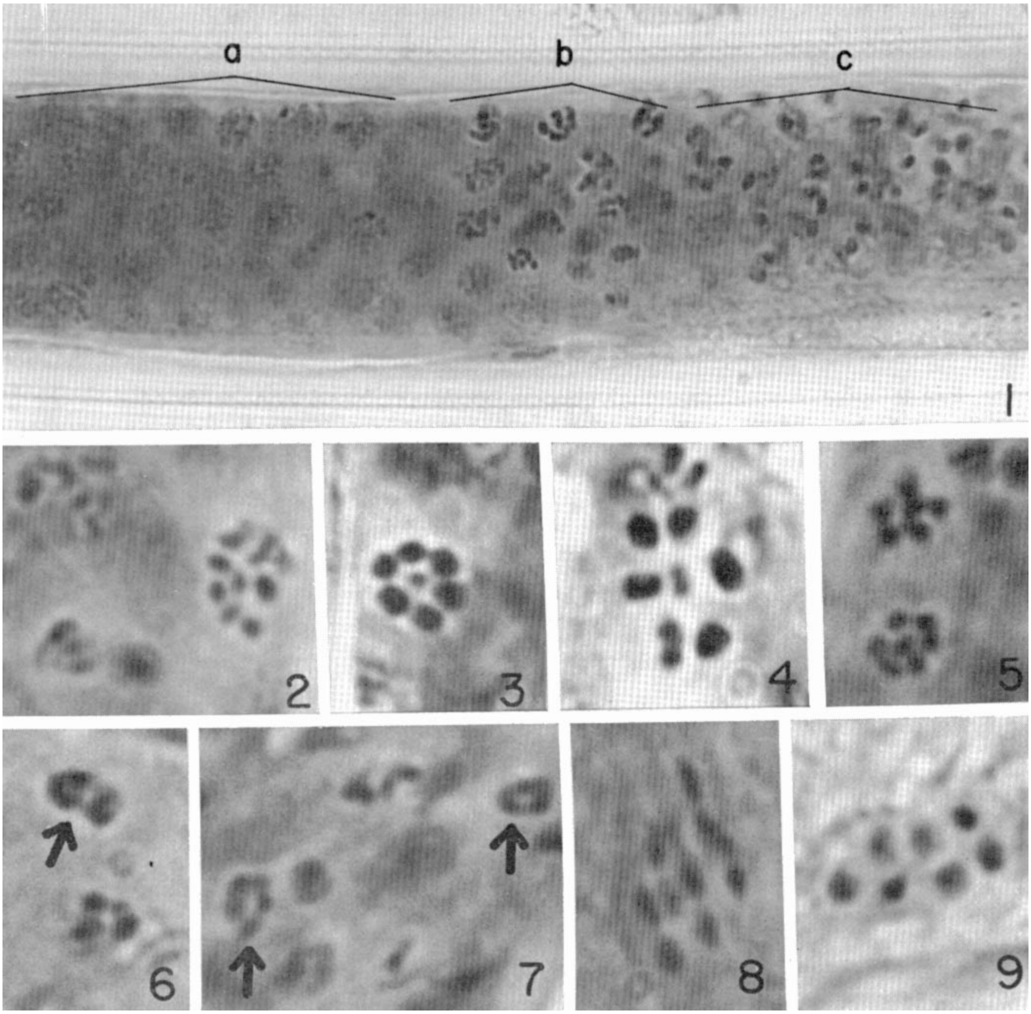
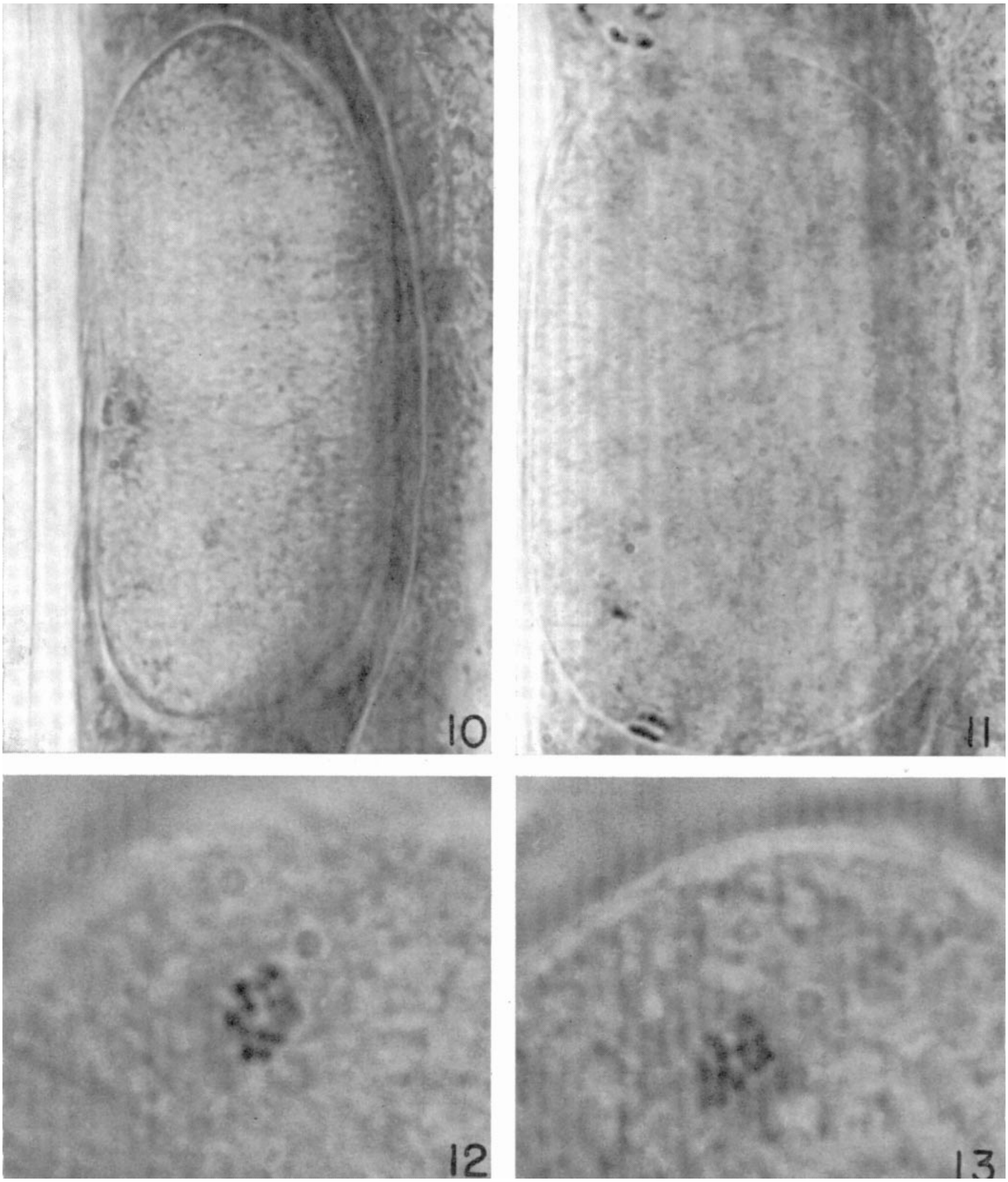


FIGS. 1–9. **1.** Portion of testis showing zone of meiotic prophase (a), zone of maturation (b), and sperm cells (c); **2.** Primary spermatocyte chromosomes in late prophase; **3.** Primary spermatocyte nucleus in first metaphase, with 6 bivalents, 1 univalent; **4.** Primary spermatocyte nucleus, first metaphase, crushed and clearly showing paired dyads; **5.** Secondary spermatocytes, second metaphase, one with 7 dyads, one with 6 (sister nuclei of these were present but not in focus); **6.** Anaphase or telophase of second meiotic division of spermatogenesis (arrow); **7.** Spermatids with ring arrangement of chromatin (arrows); **8.** Primary oocyte, just prior to the first metaphase of meiosis, 5 of the 7 bivalents visible; **9.** Polar view of metaphase, primary oocyte, 7 bivalents.

RESULTS

SPERMATOGENESIS: Mitotic divisions resulting in multiplication of spermatogonia occurred in a zone occupying only ¼ or less of the length of the testis, mostly in the distal reflexed part. The dividing chromosomes in these nuclei were small, crowded, and could not be counted.

Immediately proximal to the multiplication zone cell chromatin had a granular appearance and the nucleoli were very prominent (Fig. 1a). It was not possible to observe



FIGS. 10–13. **10.** First meiotic anaphase, oogenesis, equatorial view, nucleus near middle of egg; **11.** First meiotic anaphase of oogenesis, equatorial view, nucleus near end of egg; **12.** First meiotic anaphase of oogenesis, polar view, polar nucleus with 7 dyads; **13.** First meiotic anaphase of oogenesis, polar view, egg nucleus to Fig. 12.

the various stages of meiotic prophase in these nuclei. Just distal to the zone of meiosis, a few large nuclei were seen in which the chromatin stained intensely and the nucleoli had disappeared (Fig. 2). It was difficult to count chromosomes in these nuclei.

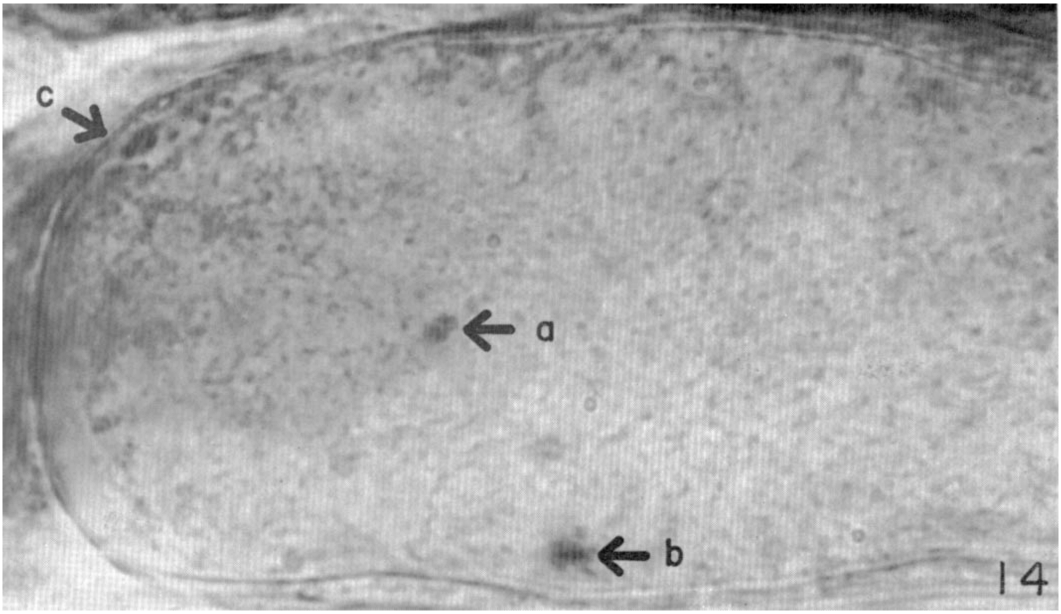


FIG. 14. Egg after second maturation division, sperm pronucleus in cytoplasm (arrow a), first and second polar nuclei, one nucleus not in focus (arrow b), third polar nucleus (arrow c), and 5 chromosomes of the 7 in the egg pronucleus near the third polar nucleus.

The zone in which the two meiotic divisions occurred was very short, consisting of 6 layers of cells or less (Fig. 1b). It was located about ⅓ the length of the testis from its anterior end. Metaphase I was the first stage at which the chromosomes could be accurately counted; 6 bivalents and 1 univalent were seen (Figs. 3, 4). The univalent was in the center, surrounded by the 6 bivalents in a circle.

At the first division one dyad from each of the 6 bivalents and the unpaired dyad passed into one daughter nucleus; the other daughter nucleus received the other 6 dyads. Therefore the nuclei at Metaphase II contained either 6 or 7 dyads (Fig. 5). At the second meiotic telophase the chromosomes were discrete but so close together (Fig. 6) accurate counts could not be made. In cells located posterior to the zone of meiosis the chromatin had coalesced into a heavily staining oval, somewhat less stained at the center (Figs. 1c, 7). Occasionally a few discrete chromosomes were visible, but they could not be counted.

All the nuclei in cells located proximal to the maturation zone were similar in appearance; the difference between spermatids and mature spermatozoa was not apparent. In younger males the mature sperm were few in number and located just proximal to the maturation zone; the remainder of the gonad was empty. In older specimens more sperm cells were present, often completely filling the entire gonad to the spicules. Sperm cells within the uterus of the female appeared the same as those in the male.

OOGENESIS: At the distal end of the ovaries the nuclei of the oogonia stained like those of the spermatogonia; nucleoli were easily visible. Mitotic divisions occurred throughout the distal ⅓ or less of the ovary. In the growth zone, proximal to the mitotic zone, neither nucleus nor nucleoli stained with orcein, and the nuclei appeared as hyaline ovals within the deeper staining, more granular cytoplasm. In oocytes which had moved from the ovary to the uterus, dark-staining chromosomes were visible at the periphery of the nucleus but were difficult to count.

At the first meiotic metaphase the nucleus was located at the periphery of the primary oocyte, with the spindle oriented perpendicular to the cell membrane. Seven bivalents were present (Figs. 8, 9). The first division usually occurred either near the middle of the oocyte (Fig. 10) or at its proximal end (toward the vulva) (Fig. 11). Only rarely was it observed at the distal end. One polar nucleus with 7 dyads was formed (Fig. 12), and 7 dyads were seen in the secondary oocyte (Fig. 13). In several eggs the first polar nucleus had divided.

The second meiotic division was seen only at the proximal end of the secondary oocyte. In one egg (Fig. 14) two polar nuclei were present at the middle, a third was present at the end, and the egg pronucleus, with 7 chromosomes, was located at the cell membrane near the third polar nucleus.

In living eggs a single polar body was seen which persisted almost until hatching time. The other two polar bodies were invisible. It was not determined which of the three polar nuclei became enclosed in a membrane as a separate polar body, nor was the fate of the other two polar nuclei observed.

In impregnated females sperm cells were located in the uterus, between the short oviduct and the vulva. Actual entry of the sperm into the developing oocyte was not seen, but sperm pronuclei were always found in the proximal part of the oocyte (toward the vulva) with its chromatin appearing either coalesced into an oval or, much more commonly, its chromosomes were discrete but small, crowded, and impossible to count.

A few oocytes, produced by females with few or no sperm in their uteri, had progressed as far as the formation of the first polar nucleus, even though no sperm cells could be found within them.

Ordinarily, cleavage begins after the egg is laid but less commonly, it may begin while the egg is still within the body of the female. Several females contained eggs with 5 or more blastomeres, and in some females several eggs in both uteri contained advanced embryos.

In both oogenesis and spermatogenesis individual chromosome morphology was similar.

ROLE OF MALES: Females isolated from other nematodes before their final molt did not produce progeny for as long as they remained alive. Some of them laid several eggs which rapidly disintegrated without undergoing cleavage. Males were placed with half of the females one week after their final molt; these females laid eggs from which larvae hatched.

DISCUSSION

Mononchoides changi reproduces amphimictically, with the classical meiotic pattern (two maturation divisions) in the production of both the female and male gametes; males are necessary for reproduction.

Three polar nuclei and the egg pronucleus are produced during oogenesis. After the second meiotic division egg shell deposition was quite advanced and the stain did not easily penetrate the egg. The cytoplasm contained many vacuoles at this stage, probably the time at which fusion of the egg and sperm pronuclei and their migration to the first cleavage plate occurred. These processes could not be seen, however, because the chromosomes would not take up the stain.

In a single observation on one egg which had been laid before it was stained, two groups of chromosomes were seen side by side in the center of the cytoplasm, one smaller than the other. This was probably the metaphase plate of the first cleavage division, with the larger group of chromosomes from the egg pronucleus, the smaller from the sperm pronucleus.

Although actual entry of the sperm into the egg was not seen, very likely the sperm entered the proximal end of the oocyte at the time the oocyte began its passage into the uterus where the sperms are stored, since the sperm pronuclei were always found in the proximal part of the oocyte.

The presence of sperm within the egg is apparently unnecessary for initiation of meiosis and egg-laying. However, cleavage and embryonation do not proceed without fertilization.

In the male the somatic chromosome number is 13; in the female it is 14. Although the chromosomes in spermatids could not be counted accurately, chromosome numbers in the secondary spermatocytes indicate that two kinds of sperm are produced, one with 6 chromosomes, the other with 7. Zygotes formed by fertilization of eggs with sperm containing 6 chromosomes become males; females receive 7 chromosomes from each parent. This XO type of sex determination also presumably occurs in *Mesodiplogaster lheritieri* (9).

Previous reports of chromosome numbers in diplogasterids (5, 6, 7, 9) seem to indicate that the basic haploid number for the group is 6. However, in *M. changi* the haploid number is 7 in females. Before generalizations about chromosome numbers in this group of nematodes can be made, many more species must be investigated.

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