# Oogenesis and Reproduction of the Birch Cyst Nematode, *Heterodera betulae*[1]

A. C. TRIANTAPHYLLOU[2]

*Abstract:* Cytological study revealed that maturation of oocytes of *Heterodera betulae* is by regular meiosis and reproduction is by parthenogenesis. Restoration of the somatic chromosome number occurs after telophase II and before egg pronucleus formation, in the absence of a mitotic apparatus through a type of endomitotic division. The haploid chromosome number is 12 ($2n = 24$) in 95% of the female nematodes studied and 13 in the remaining 5%. The phylogenetic relationship of *H. betulae* with most other *Heterodera* species having $n = 9$ is not clear. *Key Words:* Oogenesis, Reproduction, Chromosomes, *Heterodera betulae*.

The recently described *Heterodera betulae* Hirschmann and Riggs appears to reproduce in the absence of males, probably by parthenogenesis (3). A few males were found in cultures maintained at different temperature conditions (3), and a few were recovered from infected roots incubated in a moist chamber (1). To obtain more information about the mode of reproduction, the present karyological study, which also included an analysis of the meiotic cycle during oogenesis, was conducted.

## MATERIALS AND METHODS

The nematode population from which the species was originally described was used in this study (1). It was propagated on river birch seedlings, *Betula nigra* L., in the greenhouse at 20 to 30 C for more than one year before examination. Fourth stage larvae and young females obtained from infected roots were smeared on slides and stained with propionic orcein (4). Eggs from white cysts were treated 20 min with 2.5% sodium hypochlorite, smeared on slides and then stained with propionic orcein (4).

Received for publication 20 May 1970.

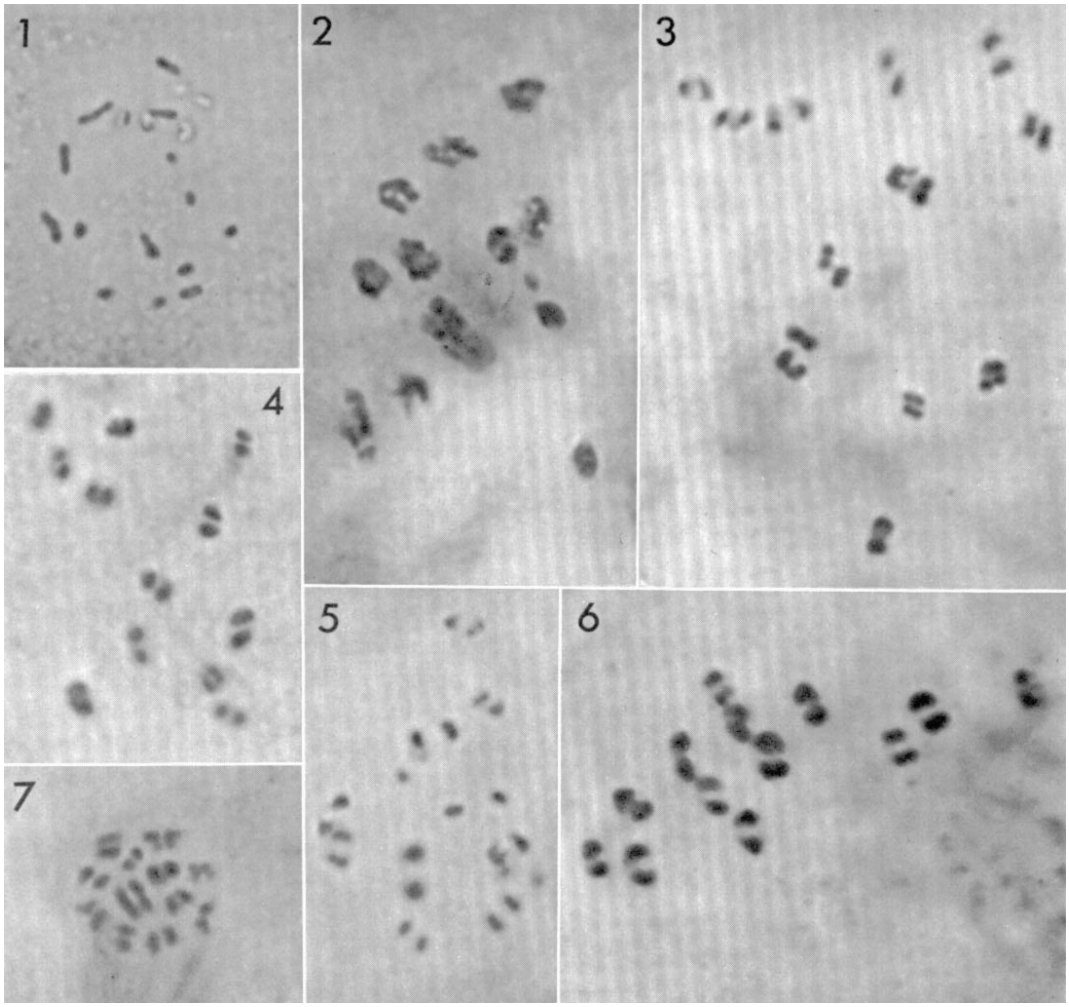
[1] Paper number 3177 of the Journal Series of the North Carolina State Agricultural Experiment Station, Raleigh, North Carolina. Supported in part by the National Science Foundation Grant (GB-7214).

[2] Department of Genetics, North Carolina State University at Raleigh, North Carolina 27607.

## OBSERVATIONS

Few oogonial divisions were observed in the germinal zone of the ovary of fourth stage larvae. The chromosome number could not be determined precisely in such divisions (Fig. 1).

Oocytes approaching the oviduct-spermatotheca area have 12 or 13 diakinetic chromosomes (Fig. 2). As the oocytes pass into the uterus, the bivalents contract and appear as tetrads (Figs. 3–6). Of the 120 females whose chromosome number was determined from prometaphase I figures, 114 had 12, and 6 had 13 bivalent chromosomes. At metaphase or early anaphase I, the two homologues of each bivalent are associated end to end, and each consists of two chromatids oriented parallel to each other (Fig. 8). The homologues move toward the opposite poles maintaining the same orientation, often with some chromatic material stretched between the separating dyads (Fig. 9). At telophase I, the chromosomes become reoriented so that they lie with the long axis of the chromatids in the same plane (Fig. 7). In the meantime, each chromosome duplicates and appears again as a tetrad (Fig. 10) similar to, but smaller than that of metaphase I chromosomes. One of the telophase plates is extruded outside the egg cytoplasm as a polar body. The second maturation division follows rapidly without an

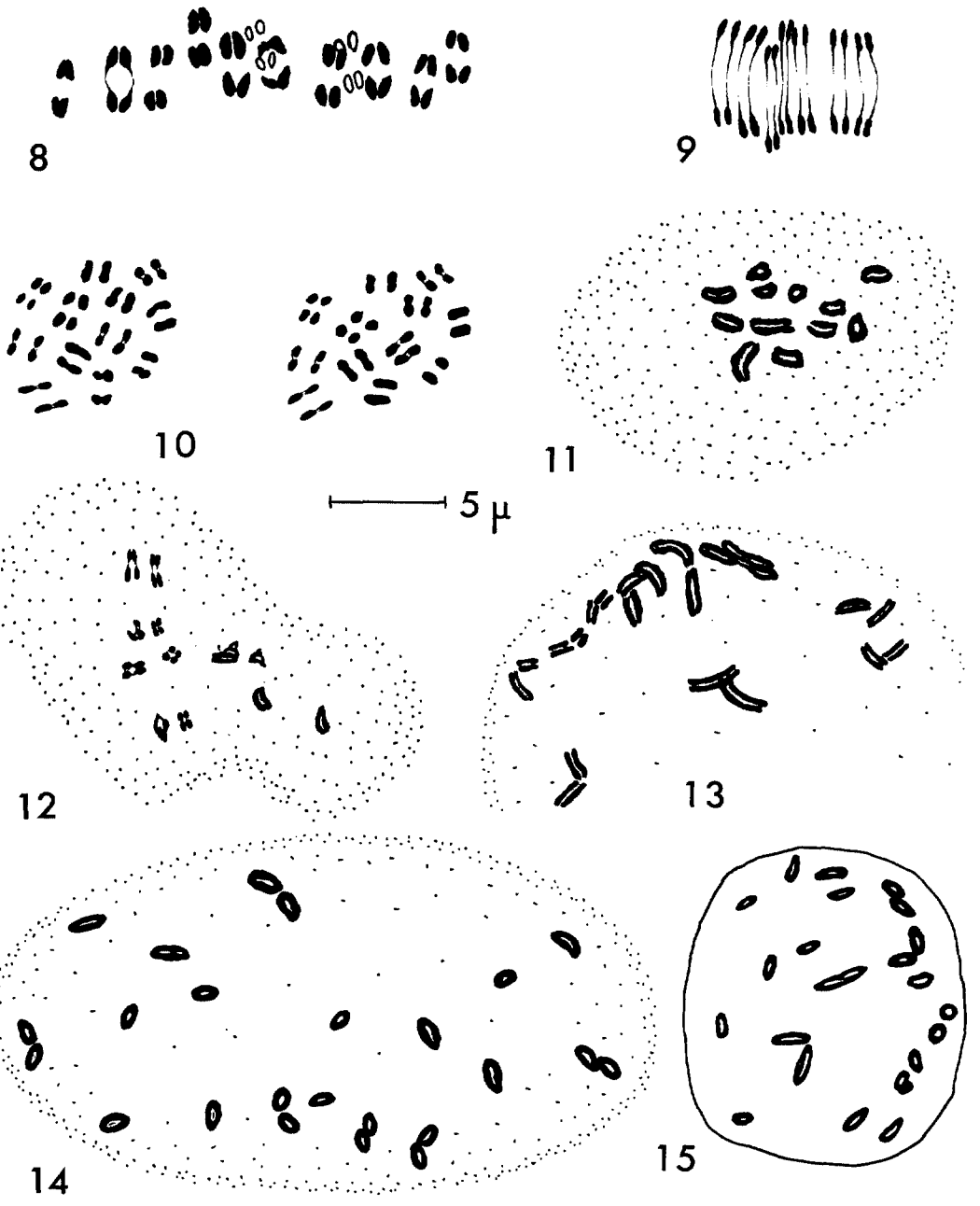



FIGS. 1–7. Photomicrographs of somatic and meiotic chromosomes during oogenesis of *Heterodera betulae* ×2600. 1. Prometaphase chromosomes of an oogonial division; 2. Thirteen diakinetic chromosomes of a primary oocyte; 3. Late prophase I figure with 12 bivalents forming tetrads; 4 and 5. Prometaphase or early metaphase I figures with 12 bivalents; 6. Prometaphase I figure with 13 bivalents; 7. Telophase I figure with 12 dyads. Duplication and formation of tetrads has started and is evident in some chromosomes.

---

→

FIGS. 8–15. Camera lucida drawings of *Heterodera betulae* chromosomes showing maturation of oocytes and restoration of somatic number. 8. Early anaphase I showing end to end orientation of homologues and side by side arrangement of chromatids; 9. Telophase I with chromatic material stretched between the separating dyads; 10. The telophase I plates of an oocyte with the earlier dyads already doubled and forming tetrads; 11. The 12 post-telophase II chromosomes in the center of the egg; 12. Doubling of the post-telophase II chromosomes; 13 and 14. Progressive stages of division of post-telophase II chromosomes in the absence of a mitotic apparatus; 15. The 24 somatic chromosomes of a blastomere (four-cell stage).


8
9
10
11
5 μ
12
13
14
15

intermediate interphase stage. When the second polar body is extruded, the first polar body is subdivided into two, and occasionally into three separate bodies, through uneven distribution of its chromosomes. Therefore, there are three to four polar bodies in mature eggs that remain visible at least up to the four-cell stage.

Following the extrusion of the second polar body, the egg chromosomes migrate to the middle of the egg cytoplasm and appear as in Fig. 11. The nucleoplasm surrounding the chromosomes is not enclosed in a nuclear envelope and is lightly stained, contrasting with the clear cytoplasm. The egg chromosomes soon duplicate again and appear as tetrads (Fig. 12). Eventually, each chromosome divides into two sister chromosomes consisting of two chromatids each (Fig. 13). There is no metaphase plate or spindle during this division and all the 24 derived chromosomes remain in the same nucleoplasmic mass. Later, the chromosomes become compact, but sister chromosomes usually remain closely associated with each other and can be recognized from nonsister chromosomes (Fig. 14). Finally, the chromosomes become diffuse and disappear, a nuclear membrane develops around the nucleoplasm and the egg pronucleus is formed.

When the chromosomes reappear in the egg pronucleus at prophase of the first cleavage, they are interconnected with each other and cannot be seen clearly or counted.

The somatic number of approximately 24 chromosomes was observed in prophase figures of the third and fourth cleavage divisions in which the chromosomes were quite distinct (Fig. 15). The homologous chromosomes, with some exceptions, were closely associated, occasionally touching each other, as if they had undergone somatic pairing. More advanced cleavage divisions were not favorable for study of the chromosomes.

## Discussion and Conclusions

Oogenesis in *H. betulae* is of the meiotic type. Synapsis appears to take place, and the haploid number of 12 and 13 bivalent chromosomes is observed at prometaphase I. Two maturation divisions occur, and two polar bodies are extruded. After telophase II and before pronucleus formation, the egg chromosomes undergo an extra duplication and a type of endomitotic division, in the absence of a mitotic apparatus. The somatic chromosome number is thus restored. No sperm was observed in oocytes, and reproduction was by meiotic parthenogenesis. However, the presence of occasional males under certain environmental conditions (1, 3) and the meiotic type of maturation of the oocytes suggest that cross fertilization may occur under certain circumstances.

Two forms, with 12 and 13 chromosomes, were present in the population studied. The form with 13 chromosomes constituted 5% of the adult population in 1 to 2-year-old greenhouse cultures, but was less frequent in 4-year-old cultures. It is possible that this chromosomal form was reduced progressively under greenhouse conditions and that it may occur at frequencies higher than 5% under natural conditions.

Maturation of oocytes in females with 13 chromosomes appeared to be normal. Tripolar spindles were observed in some cleaving eggs, but it could not be determined whether such eggs had 24 or 26 chromosomes. Giant eggs, slightly less than twice the size of normal eggs had approximately 40 to 50 chromosomes, but none had developed beyond the two-cell stage. Similar nonviable, polyploid giant eggs are common in many *Heterodera* and *Meloidogyne* species and appear to develop as a result of the inclusion of two nuclei in the same cytoplasmic mass during a premeiotic division such as the last oogonial division. The presence of small anucleate oocytes often observed close to the

giant eggs in microscopic preparations supports this view. Apparently, these small oocytes have contributed the nucleus and part of their cytoplasm to the oocytes that eventually develop into giant eggs. Furthermore, viable giant and small-size eggs are common in these organisms and hatch into giant and small larvae, respectively. Such viable eggs probably are derived occasionally as a result of an unequal distribution of the cytoplasm during the last oogonial division. They always have a nucleus with a normal chromosomal complement. Recent experiments with regard to the incidence and heritability of similar aberrant forms in *Heterodera schachtii* appear to be in agreement with this interpretation based on cytological criteria (2).

*H. betulae* is morphologically distinct among known *Heterodera* species (1). The present study demonstrated that it is also cytologically distinct, having n = 12 and 13 chromosomes as compared to n = 9 of all other *Heterodera* species that undergo meiosis. The closest morphological relatives of *H. betulae* are *H. cacti* and *H. weissi* (1). Of those, *H. cacti* has not been studied cytologically, but *H. weissi* has n = 9 and reproduces by amphimixis (unpublished information). At present, it is difficult to relate *H. betulae* phylogenetically with any other species of *Heterodera.*

## LITERATURE CITED

1. HIRSCHMANN, HEDWIG, AND R. D. RIGGS. 1969. *Heterodera betulae* n. sp. (Heteroderidae), a cyst-forming nematode from river birch. J. Nematol. 1:169–179.
2. JOHNSON, RUTH N., AND D. R. VIGLIERCHIO. 1970. Incidence of aberrancy in *Heterodera schachtii.* Nematologica 16:33–38.
3. RIGGS, R. D., HEDWIG HIRSCHMANN, AND M. L. HAMBLEN. 1969. Life cycle, host range, and reproduction of *Heterodera betulae.* J. Nematol. 1:180–183.
4. TRIANTAPHYLLOU, A. C., AND HEDWIG HIRSCHMANN. 1966. Gametogenesis and reproduction in the wheat nematode, *Anguina tritici.* Nematologica 12:437–442.