Oogenesis and the Chromosomes of Twelve Bisexual Species of Heterodera (Nematoda: Heteroderidae)[1]

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Abstract: Twelve bisexual species of *Heterodera* reproduced by amphimixis and had the same number of n=9 (2n=18) chromosomes in maturing oocytes. *H. schachtii* had slightly larger chromosomes than all other species. Only sperm nuclei with n=9 chromosomes were observed inside maturing oocytes and no specialized sex chromosomes were detected in any case. A "supernumerary" chromosome was observed occasionally in oocytes of *H. schachtii* and *H. weissi* and was transmitted regularly to one-half of the progeny of the nematodes that possessed it. Cytological characteristics were not very instructive in differentiating amphimictic *Heterodera* species. Such karyotypic uniformity indicates cytogenetic stability of the genus and close interrelationship among its members.
Key Words: reproduction, supernumerary chromosomes, chromosome number, cyst nematodes.

Information about chromosome numbers, type of maturation of gametocytes and mode of reproduction of 20 species of *Heterodera* A. Schmidt is available (7). Some of this information, however, is reported as preliminary observations or unpublished data. With the exception of *H. betulae* Hirschmann and Riggs, which has haploid chromosome numbers of 12 and 13 (6), and *H. rostochiensis* Wollenweber which reportedly exhibits a variation of n=9, 10, 11, and 23-24 chromosomes (1, 2), all other species of *Heterodera* have a haploid number of n=9 chromosomes. Most species are amphimictic,

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diploid, but some are parthenogenetic and exhibit various degrees of polyploidy (7). An evaluation of the cytogenetic situation within the entire genus could be helpful in understanding phylogenetic relationships of its members. With this objective in mind, I compared twelve bisexual species of *Heterodera* and attempted to detect differences or similarities in their cytology.

MATERIALS AND METHODS

Nematode populations (Table 1) were obtained during the last ten years and maintained on host plants in a greenhouse at 22-28 C. Most were studied soon after they were obtained, and at 2-year intervals thereafter. White egg-producing females from 30- to 40-day-old greenhouse cultures were smeared on chemically cleaned microscope slides, which were then submerged for 5 min in 1 N HCl and for 25 min in 3:1 (v/v) ethyl alcohol-acetic acid mixture. Following fixation two drops of 2% propionic orcein were applied to each smear, which was then covered with the depression of an inverted cavity slide to prevent rapid evaporation of the stain. After 20 min, the stain was drained, and the entire slide was submerged for 5 sec in 40% propionic acid to remove excess stain. A

TABLE 1. Nematode populations included in this study of chromosome numbers in selected *Heterodera* spp.

Nematode species	Population (designation)	Source	Host plant
Heterodera schachtii A. Schmidt	27-Engl	England	Red beet *(Beta vulgaris)*
Heterodera schachtii	39-Can	Canada	Red beet
Heterodera schachtii	99-Wisc	Wisconsin-USA	Red beet
Heterodera schachtii	139-Holl	Holland	Red beet
Heterodera schachtii	326-Ger	Germany	Red beet
Heterodera leuceilyma Di Edwardo & Perry	320-Fla	Florida-USA	St. Augustinegrass *(Stenotaphrum secundatum)*
Heterodera cruciferae Franklin	143-Holl	Holland	Cabbage *(Brassica oleracea)*
Heterodera cruciferae	147-Engl	England	Cabbage
Heterodera cruciferae	240-Engl	England	Cabbage
Heterodera cruciferae	316-Cal	California-USA	Cabbage
Heterodera carotae Jones	32-Engl	England	Carrot *(Daucus carota)*
Heterodera carotae	142-Holl	Holland	Carrot
Heterodera carotae	243-Engl	England	Carrot
Heterodera fici Kirjanova	321-Va	Virginia-USA	Fig *(Ficus carica)*
Heterodera graminophila Golden & Birchfield	309-La	Louisiana-USA	Barnyard grass *(Echinochloa colonum)*
Heterodera longicolla Golden & Dickerson	333-Kan	Kansas-USA	Buffalo grass *(Buchoë dactyloides)*
Heterodera tabacum Lownsbery & Lownsbery	152-Mass	Massachusetts-USA	Wonderberry *(Solanum burbankii)*
Heterodera solanacearum Miller & Gray	148-Va	Virginia-USA	Tobacco *(Nicotiana tabacum)*
Heterodera virginiae Miller & Gray	149-Va	Virginia-USA	Tobacco
Heterodera virginiae	276-NC	N. Carolina-USA	Tobacco
Heterodera sp. (Mexican cyst nematode)[a]	261-Mex	Mexico	Tobacco
Heterodera weissi Steiner	8-NC	N. Carolina-USA	Smartweed *(Polygonum pennsylvanicum)*
Heterodera weissi	50-Md	Maryland-USA	Smartweed
Heterodera weissi	51-Wisc	Wisconsin-USA	Smartweed
Heterodera weissi	155-Va	Virginia-USA	Smartweed
Heterodera weissi	319-Ill	Illinois-USA	Smartweed
Heterodera weissi	318-Ark	Arkansas-USA	Smartweed

[a]This nematode has been described as *Heterodera mexicana,* but only in a Ph.D. thesis. [A. Campos Vella. 1967. Taxonomy, life cycle and host range of *Heterodera mexicana,* n. sp. (Nematoda, Heteroderidae). Ph.D. Thesis, University of Wisconsin. 70 p.]

coverslip was applied and sealed with a 1:1 mixture of paraffin and lanolin. Such temporary preparations were examined within a few days, although they remained in good condition for 1-2 months.

To study advanced stages of maturation, fertilization and cleavage divisions, eggs obtained by dissecting gravid females were treated for 10-20 min in 2% sodium hypochlorite and washed for 30 min in distilled water. This treatment made the egg membranes permeable to the fixative and stain. Eggs were later transferred to a 10% plain gelatin solution, and the suspension spread thinly on slides and left to dry for a few minutes till the gelatin had solidified. Then the slides were submerged in 1 N HCl and processed further into fixative and stain as described for the nematode smears.

OBSERVATIONS

Oogenesis and fertilization: The female gonad of all species studied is similar to that of *H. glycines* Ichinohe described and illustrated earlier (8). Oogonial divisions occur in fourth-stage and fourth-molt larvae, as well as in very young females, but such divisions are not favorable for cytological analysis, because the chromosomes are usually entangled with each other and therefore not discrete (Fig. 1). In a few exceptional cases, 18 distinct prometaphase chromosomes were observed in oogonia of *H. virginiae* (149-Va) and *H. graminophila* (309-La).

Behavior of chromosomes during the first and second maturation division, and the process of fertilization of all species studied, follow the same pattern as described for *H. glycines* (8). Nine bivalent chromosomes were observed at prophase I in oocytes approaching the spermatotheca, and at prometaphase or metaphase I in oocytes located in the proximal region of the uterus (Fig. 2-5, 14-16). Each bivalent consists of two univalent chromosomes which are characteristically associated end to end (Fig. 3, 4, 16). Each univalent consists of two rod-shaped chromatids which have been precociously separated from each other and are arranged side by side. Each bivalent, therefore, is tetrapartite (tetrad) with the four components representing four individual chromatids rather than chromosome arms, as they would in the case of metacentric prometaphase chromosomes of other animals. At metaphase I, the bivalents condense further and, most often, the chromatids are not clearly discernible (Fig. 5, 15).

The prometaphase chromosomes of *H. schachtii* vary in length from 1.6 to 2.4 μm. This includes the lengths of the two univalents and a 0.4 μm-space between them. All other species studied have slightly smaller chromosomes, varying in length from 1.4 to 1.9 μm. On the basis of chromosome size the chromosomal complement of *H. schachtii* can be distinguished from those of the other species, but not consistently. Differences in the degree of condensation of prometaphase chromosomes in different preparations often make such a distinction difficult.

The best stage for characterizing the chromosomes of each chromosomal complement is telophase I, especially the telophase plate that forms the first polar nucleus. The chromosomes of the first polar nucleus maintain their position in the telophase plate for a long time and can often be observed in polar view, showing several structural details (Fig. 6, 7, 8, 17, 18). Each chromosome consists of two chromatids that lie parallel to each other. The chromatids are rod-shaped and the longest of them often have club-shaped ends with a distinct constriction in the middle of their length. The two smallest chromosomes of *H. schachtii,* with chromatids 0.6 - 0.7 μm long, can usually be distinguished from the remaining chromosomes which vary from 0.9 to 1.8 μm in length (Fig. 6, 7, 17). Several small chromosomes of the *H. tabacum* species group have almost spherical chromatids 0.4 μm in diameter, whereas the large chromosomes have rod-shaped chromatids, 0.3 - 0.4 μm wide and 0.8 - 1.0 μm long (Fig. 18).

At metaphase II, the chromosomes are oriented on the metaphase plate with the chromatids parallel to each other and facing the opposite spindle poles (Fig. 9). At anaphase II the chromatids move toward the poles "broad side front" and show no signs of localized kinetochore activity, although often, the chromatid ends appear to be bent toward the spindle poles (Fig. 19).

One spermatozoon enters each oocyte, as the oocytes at prometaphase pass through the spermatotheca, and remains unchanged at one pole of the oocyte till completion of both maturation divisions. The chromosomes of

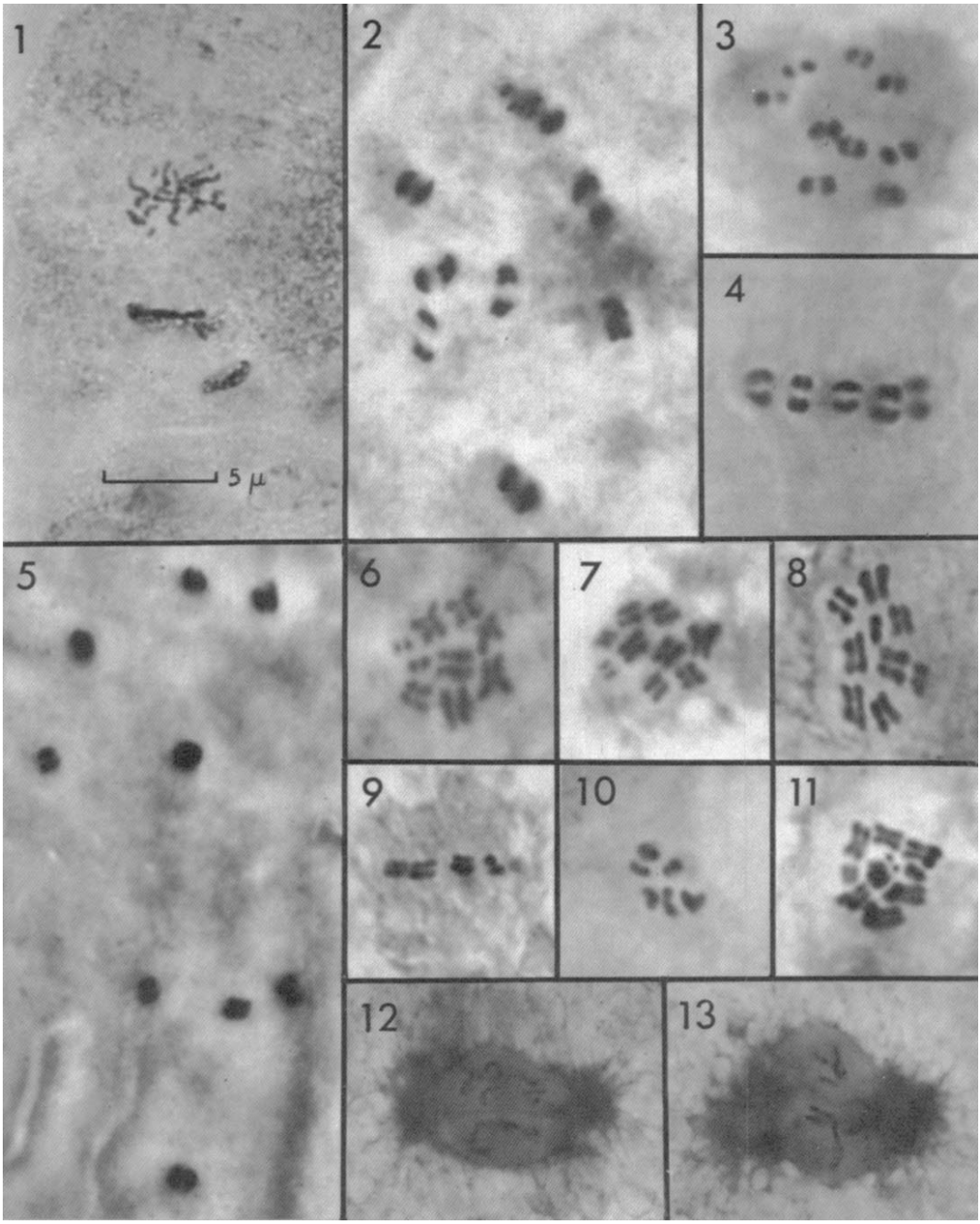

FIG. 1-13. Photomicrographs of chromosomes of various *Heterodera* species during oogenesis and fertilization. **1)** Oogonial divisions in the apical region of the ovary of a young female of *H. carotae* (population 142-Holl). The individual chromosomes are not discernible. **2, 3)** Prometaphase figures in primary oocytes of *H. schachtii* (27-Engl) and *H. solanacearum* (148-Va), respectively, with nine bivalent chromosomes each. **4)** Side view of a metaphase I chromosomal figure of *H. schachtii* (39-Can). **5)** Metaphase I in an oocyte of *H. schachtii* (27-Engl) with highly condensed chromosomes. **6, 7, 8)** Telophase I chromosomes from three oocytes of *H. schachtii* (39-Can) showing details of chromatid structure and variation among telophase figures. The two smallest chromosomes are distinguishable from the others. **9)** Metaphase II in an oocyte of *H. schachtii* (27-Engl). **10)** The sperm nucleus inside an oocyte of *H. schachtii* (39-Can) with discernible chromosomes. Usually the chromosomes of the sperm nucleus are interconnected and not discrete. **11)** Telophase I in an oocyte of *H. schachtii* (39-Can) with a very small supernumerary chromosome in addition to the regular chromosomal complement. **12)** The zygote nucleus shortly after fusion of egg and sperm pronuclei in *H. schachtii* (27-Engl). **13)** Same as in 12, but a little later, when the nucleus prepares for the first cleavage division. Scale for Fig. 2-13 as in Fig. 1.

the sperm nucleus are clumped together in one or few interconnected masses, but in some cases the chromatin is distributed in distinct bodies, most of which appear to represent individual chromosomes (Fig. 10). Sperm nuclei with discernible chromosomes were observed frequently in oocytes of *H. weissi* and occasionally in *H. schachtii* and *H. cruciferae*. Nine spherical chromosomes were counted in a least ten sperm nuclei of *H. weissi*, suggesting that only one kind of sperm, with nine chromosomes, is produced by this species. In two exceptional cases observed in *H. schachtii* and *H. cruciferae* nine rod-shaped chromosomes were clearly observed in the sperm nucleus at a period when the egg nucleus was at metaphase II (Fig. 20).

Following the second maturation division, the sperm and egg pronuclei are formed. They soon approach each other in the center of the egg and fuse to form the zygote nucleus. Shortly before fusion, the chromosomes condense and become visible as long threads in both pronuclei. At the same time, two

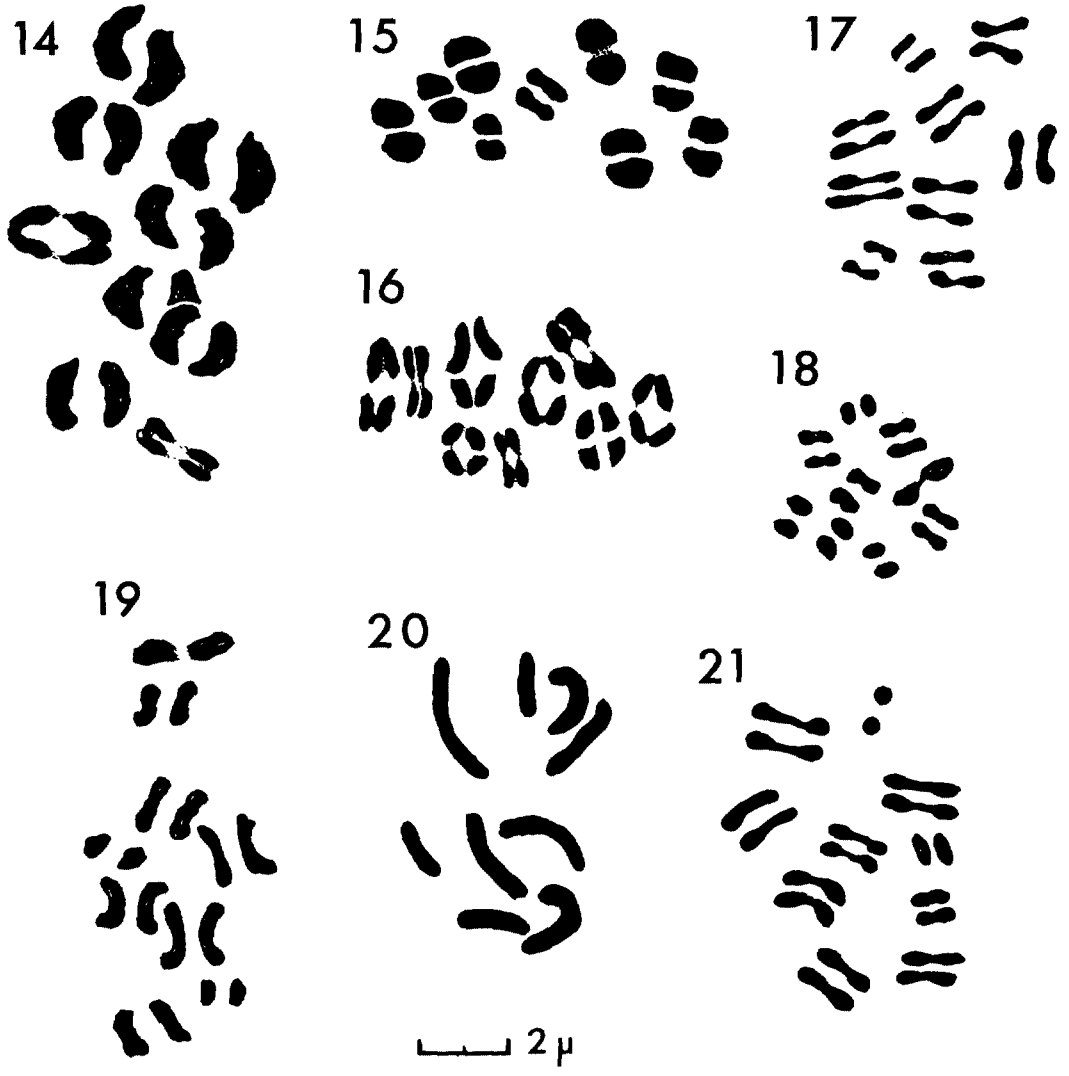

FIG. 14-21. Camera lucida drawings of the chromosomes of various *Heterodera* species. **14, 15, 16**) Prometaphase chromosomes in primary oocytes of *H. schachtii* (population 99-Wisc), *H. virginiae* (149-Va) and *Heterodera* sp. (216-Mex), respectively. **17, 18**) Telophase I chromosomes in oocytes of *H. schachtii* (99-Wisc) and *H. virginiae* (149-Va), respectively. **19**) Early anaphase II in a secondary oocyte of *H. schachtii* (139-Holl). **20**) Chromosomes of a sperm nucleus observed in a secondary oocyte of *H. schachtii* (39-Can). **21**) Telophase I in an oocyte of *H. schachtii* (39-Can) with a small supernumerary chromosome in addition to the regular complement.

centrosomes become visible in opposite sides of the fusing pronuclei and on a line parallel to the long axis of the egg (Fig. 12). Soon the chromosomes condense further and become oriented on a metaphase plate preparing for the first cleavage division (Fig. 13). No definite counts of the somatic chromosomes during cleavage divisions could be made.

Variation in chromosome numbers: A small number of females of populations 27-Engl and 39-Can of *H. schachtii* had oocytes with an extra chromosome in addition to the nine bivalents commonly observed at metaphase I. About 50% of the progeny of such females had this extra chromosome. At metaphase I, the extra chromosome was univalent, consisting of two spherical chromatids, 0.4 μm in diameter, about 0.4 μm apart from each other. The chromatids did not separate at anaphase I, but the entire chromosome passed, apparently with the same frequency, either toward the polar or the egg nucleus (Fig. 11, 21). Its fate beyond telophase I could not be followed, but its presence in approximately one-half of the progeny indicates that it divided normally during the second maturation division. No phenotypic effects could be associated with the presence or absence of this chromosome and its role or function is not known. It can be regarded as a "supernumerary" chromosome like those described in many species of animals and plants (9). An extra chromosome was also observed in *H. weissi* (319-I11), in four oocytes from the same female. Three of the oocytes were at metaphase I and had nine bivalent and one univalent chromosomes. The fourth oocyte was at metaphase II and its first polar nucleus had ten chromosomes.

DISCUSSION AND CONCLUSIONS

Oogenesis and the process of fertilization of all *Heterodera* species included in this study follow the same pattern described earlier for *H. glycines, H. oryzae* (Luc and Berdon-Brizuela), and other amphimictic, plant-parasitic nematodes (5, 7, 8). The chromosomal complement of *H. schachtii* is indistinguishable from that of *H. glycines*. The chromosomal complements of all other species are similar to that of *H. schachtii,* but the chromosomes are slightly smaller. Work in progress shows that observed differences in chromosome size are correlated with differences in the amount of DNA present in the sperm nucleus. *Heterodera schachtii* and *H. glycines* which have chromosomes of the same size also have the same DNA content (4).

The chromosomes of the sperm nucleus in oocytes of *H. weissi* were all small and spherical, whereas those of *H. schachtii* and *H. cruciferae* were larger and most of them rod-shaped. Part of this difference undoubtedly is due to the degree of condensation of the chromatin. Apparently in *H. weissi* the chromosomes of the sperm nucleus become very compact, and for this reason, are discrete in approximately 5% of the oocytes, whereas this happens very rarely in other species.

The supernumerary chromosomes in *H. schachtii* and *H. weissi* appeared to have no phenotypic effects and their significance, if any, is not understood. However, it shows that certain cytological anomalies occur in these organisms and could be the cause of variation in chromosome numbers. *Heterodera* species with chromosome numbers greater than nine are known (7).

No sex chromosomes were distinguished in the female karyotype of the various species and only sperm with nine chromosomes were observed in oocytes of *H. weissi.* Therefore, if there is indeed a chromosomal mechanism of sex determination in these organisms, it must be of the XX-XY type with the X and Y chromosomes being indistinguishable from each other and from the autosomes. Most other nematodes with a known chromosomal mechanism of sex determination are of the XX-XO type (7).

The cytological information presented here is of little value in differentiating amphimictic species of *Heterodera*. Except for some differences in chromosome size, the karyotypes of the various amphimictic species are indistinguishable. This karyotypic uniformity suggests cytogenetic stability of the genus and close phylogenetic relationships, especially among species of the same species group. Furthermore, it explains some interbreeding that apparently is possible among related *Heterodera* species (3).

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