Polyploidy in an Amphimictic Population of Heterodera glycines[1]

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Abstract: A tetraploid single-cyst isolate of *Heterodera glycines* from a field population from Indiana has been propagated in the greenhouse on Lee soybeans since its discovery, in 1973. The tetraploid isolate has n = 18 chromosomes, compared with n = 9 of the diploid *H. glycines;* it has larger cysts and larvae, but shows the same level of parasitism and host range as the diploid population from which it apparently evolved. Association of chromosomes is irregular at metaphase I, with quadrivalents, trivalents, and univalents often observed in addition to the bivalents. The second maturation division is usually normal. About 80% of the mature oocytes (just before fertilization) have n = 18, and the other 20% have n = 17 or 19. Reproduction of the tetraploid isolate is exclusively by cross-fertilization. The discovery of such a tetraploid provides an experimental tool for the study of polyploidy in nematodes. Many amphimictic plant-parasitic nematodes are suspected of representing polyploids. *Key Words:* soybean cyst nematode, cross-fertilization.

Polyploidy has played a major role in the evolution of higher plants, especially by facilitating the establishment of allopolyploids, i.e., fertile polyploids of hybrid origin (6, 11). More than one-third of all species of plants are estimated to have arisen through polyploidy. In animals, polyploidy is encountered frequently among parthenogenetic species, whereas in cross-fertilizing species it appears to be rare or, according to many authors, does not occur at all. Muller, in 1925, reasoned that polyploidy in bisexual species of animals upsets their sex chromosome mechanism and therefore cannot be successful (4). Although several other explanations have been proposed since then, imbalance in sex determination is still the most accepted explanation of the rarity of polyploidy among animals. According to Mayr (3), "most alleged cases of polyploidy in animals are based on a misinterpretation of cytological evidence ('pseudopolyploidy') or are limited to forms that lack gametic sex determination (such as hermaphrodites), or that reproduce uniparentally (parthenogenesis)." Furthermore, Mayr implies that if polyploidy exists in amphimictic animals, it may have been established during a parthenogenetic stage in their evolution, or it occurs in animals in which sex is determined by a definite sex gene rather than by a balance of sex-determining factors. Similarly, Astaurov supported that polyploidy in bisexual species of animals is rare and probably has an indirect origin via polyploid parthenogenesis and secondary reversal to bisexuality (1). He also expressed the opinion that the sterility of autotetraploids is probably a more serious obstacle to the establishment of bisexual polyploidy than the imbalance of the sex-determining mechanism. White, in 1973, concluded that there may be a few genuine cases of evolutionary polyploidy in bisexual animals, but the topic is one that deserves further consideration with objectivity and critical judgment (11).

The present article deals with an apparent case of polyploidy in a plant-parasitic nematode which reproduces by cross-fertilization. In 1973, the junior author observed that larvae hatched from a cyst of *Heterodera glycines* were distinctly larger

Received for publication 17 April 1979.
[1] Paper No. 5912 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. This study was supported in part by National Science Foundation Grant No. DEB. 76-20968 A02.
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than larvae from other cysts of the same field population. About 100 large larvae from that special cyst and about 2,000 regular-size larvae from 10 other cysts of the same population were isolated and propagated separately on two soybean plants. Later, preliminary examination of larvae and females revealed that: a) larval size was stable and characteristic of each isolate; b) both isolates reproduced by cross-fertilization; c) females of the isolate with large larvae had about twice as many chromosomes as those of the isolate with larvae of regular size. These observations suggested the existence of polyploidy in a cross-fertilizing nematode, a unique case that deserved further consideration. The present comparative study of the cytogenetic, morphometric, and host-range characteristics of the two isolates confirms the polyploid amphimictic nature of the isolate with the large larvae. Further investigations are under way concerning the implications of polyploidy combined with amphimixis in this nematode.

MATERIALS AND METHODS

The population of *H. glycines* used was obtained in 1973 from a soybean field in Vanderburgh County, Indiana. About 100 large-size larvae hatched from one cyst were used to inoculate a two-week-old soybean seedling (*Glycine max* Merr. cv Lee). Another 2,000 regular-size larvae obtained from 10 cysts of the same population were used to inoculate a second soybean seedling. The two isolates thus established were propagated separately on Lee soybeans for the following 5 years.

For the cytological study, egg-laying females were processed and stained with propionic orcein as described for other *Heterodera* species (8).

To test for the capacity of females to reproduce in the absence of males, single-larva inoculations were made of soybean seedlings germinated in sand in plastic petri dishes. Four days after inoculation, the seedlings were transplanted in 10-cm-diam pots filled with a 1:1 mixture of sand and soil. Forty-five days after transplanting, the roots were examined for females, with or without egg masses. Some of the females found were stained with propionic orcein and examined for eggs inside the uterus and the state of development of the eggs. The rest of the females were kept for 3 weeks in a moist chamber and periodically examined for the production of embryonated eggs or for hatched larvae.

For the morphometric study, 50 yellow females (cysts) were obtained from stock cultures of each isolate, and the length and width of each were measured. The cysts were then broken to release the eggs and larvae. Four larvae were picked at random from each of 25 cysts from each isolate, mounted on slides in water, and heated gently to relax the larvae for easier measurement. Females were measured at 25×, larval length at 100×, and other morphometrics (Table 1) at 1,000×.

The host-range study was conducted in a greenhouse at 25–30 C. The source of inoculum was cultures maintained in an actively reproducing condition. Females and cysts were obtained by the roiling and sieving method and placed in a Waring blender to release the eggs and larvae. The suspension of eggs and larvae was injected into the soil in each pot with an automatic pipette which delivered a uniform amount (about 2,000 eggs and larvae) to each pot. Some plants were germinated in the testing medium, whereas others were transplanted from a stock source. Five weeks after inoculation the females and cysts were recovered separately from each plant by the roiling and sieving method, and counts were made using a stereomicroscope. The counts were converted to an index figure (index of parasitism) by dividing the number recovered from each plant by the average number recovered from the Lee soybean cultivar and multiplying by 100.

OBSERVATIONS

Cytology and mode of reproduction: The cytological behavior of the isolate with regular-size larvae (diploid isolate) was identical to that described for *Heterodera glycines* (9). Nine bivalent chromosomes were observed in metaphase I (Fig. 1), and nine univalents in telophase I, polar body I, and metaphase II figures (Fig. 3).

The general pattern of maturation of oocytes of the isolate with large larvae (tetraploid isolate) was also similar to that

TABLE 1. Egg counts and morphometrics of a diploid and a tetraploid isolate of *Heterodera glycines*.

Character	Diploid isolate	Tetraploid isolate
1) Larval body length (100)[a]	400 μm ± 12.9[b]	642 μm ± 21.5
2) Larval body width (100)	20.8 μm ± 0.6	23.2 μm ± 1.1
3) Larval stylet length (100)	22.6 μm ± 0.3	27.8 μm ± 0.7
4) Larval tail, clear portion (100)	25.3 μm ± 1.3	36.4 μm ± 2.2
5) Larval DGO[c] (100)	4.6 μm ± 0.3	8.4 μm ± 0.7
6) Cyst length (25)	761 μm ± 20.0	891 μm ± 20.1
7) Cyst width (25)	521 μm ± 13.9	527 μm ± 13.9
8) No. of eggs in body (25)	112 ± 6.4	126 ± 4.3
9) No. of eggs in egg mass (25)	7 ± 2.1	5 ± 3.3

[a]Number of observations.
[b]Mean ± standard error of the mean.
[c]Distance of dorsal esophageal gland orifice from stylet.

described for *H. glycines* (9). However, the chromosome number in metaphase I figures was difficult to determine because of irregular association of the chromosomes. In some metaphase I figures, 18 bivalent chromosomes could be counted (Fig. 2), whereas in most figures the number of chromosomes seen was variable (12–17). Some bivalents, some univalents, and some with rather complex configurations were present, apparently representing trivalents and quadrivalents. The most favorable for chromosome counts were telophase I and metaphase II figures. Usually 18 chromosomes were counted in the first polar body nucleus or telophase I and metaphase II figures (Fig. 4), although 17 or 19 were counted in about 20% of the 90 figures scored (Figs. 6, 7). In three oocytes from different females where both telophase plates could be counted precisely, one plate had 17 chromosomes and the other 19. Another five oocytes had 18 chromosomes in both telophase plates.

At telophase I and metaphase II, all the chromosomes were univalents, each consisting of two distinct chromatids oriented parallel to each other (Fig. 5). The size and morphology of the chromosomes were similar to those of the diploid isolate.

Sperm was always present in the spermatotheca of egg-producing females of both isolates, and a sperm nucleus was always present in oocytes that had advanced beyond metaphase I. The chromosomes of the sperm nucleus could not be counted precisely, but more than 9, and often about 18, chromosomes could be seen in the tetraploid isolate, especially during the second maturation division of the oocytes, when the sperm nucleus chromosomes tended to become more discrete.

After the second maturation division, the egg and sperm pronuclei were formed and fused to produce a zygote nucleus. Reproduction was thus by amphimixis in both isolates.

Amphimictic reproduction was also demonstrated in the tetraploid isolate by inoculating each of 50 soybean seedlings with a single second-stage larva. One virgin female nematode was recovered in each of six plants 45 days after inoculation, and none had produced any eggs or larvae. Inseminated females recovered from plants inoculated with 500 larvae each produced eggs within 25 days of inoculation.

DNA content: The relative amount of DNA in sperm nuclei obtained from the seminal vesicle of males was determined microspectrophotometrically with the two-wave-length method (2, 5). The control for comparison was the diploid isolate. The DNA content of sperm nuclei of the polyploid isolate was 155.6 relative units, compared with 100 units for the diploid isolate. This value is lower than the 200 units expected for a tetraploid, and possibly indicates a loss of some DNA following polyploidization. These are approximate measurements, however, because of the difficulty of measuring DNA of spermatozoa, which is highly condensed in a small spherical area, allowing minimal light transmission.

Morphometrics: Morphometric charac-

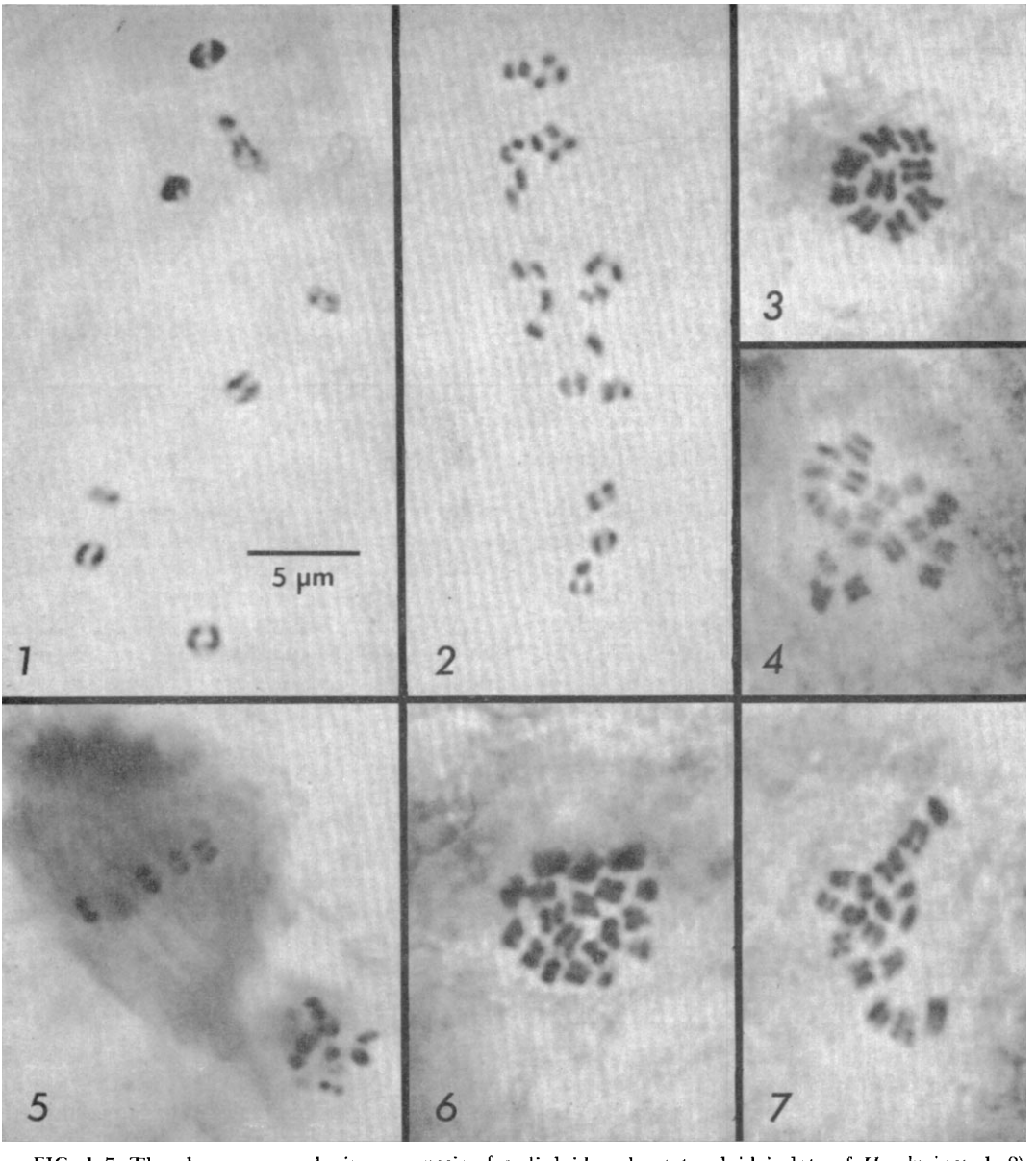

FIG. 1–7. The chromosomes during oogenesis of a diploid and a tetraploid isolate of *H. glycines*. 1, 2) Respectively metaphase I chromosomes of the diplond (n = 9) and the tetraploid (n = 18) isolates. 3, 4) Respectively first polar nuclei of the diploid and the tetraploid isolates, with 9 and 18 chromosomes. 5) Metaphase II of the tetraploid isolate showing univalent chromosomes and normal dissociation of the chromatids. First polar nucleus visible in the lower right corner. 6, 7) Respectively metaphase II and polar nucleus I of the tetraploid isolate with 19 and 17 chromosomes, resulting from irregular first maturation division. Scale in all figures as given in Fig. 1.

teristics clearly differentiate the tetraploid from the diploid isolate (Table 1). The tetraploid isolate is characterized by longer measurements of larvae and cysts. About the same number of eggs were found in cysts of both isolates.

Host range: Host-range tests involving 37 test plants inoculated with a suspension of 2,000 eggs and larvae failed to show any substantial differences between the two isolates in host range and level of parasitism (Table 2).

DISCUSSION

The present study indicates that the isolate with the large larvae is a tetraploid of

TABLE 2. Development of a diploid and a tetraploid isolate of *Heterodera glycines* on various plants.

Host plant[a]		Index of parasitism[b]	
Scientific name	Cultivar	Diploid isolate	Tetraploid isolate
1) *Lespedeza stipulacea*	Korean	23	23
2) *L. striata*	Kobe	110	72
3) *Melilotus officinalis*		0.2	2
4) *Cleome* sp.	Pink Queen	40	21
5) *Robinia pseudoacacia*		1	0
6) *Echinochloa cristagalli*		0	0.1
7) *Polygonum pennsylvanicum*		0.1	0
8) *Glycine max*	Lee	100	100
9) *Glycine max*	Pickett	2	1
10) *Glycine max*	Peking	0.5	0.2
11) *Glycine max*	Old Dominion	17	19
12) *Glycine max*	Pine Dell Perfection	25	14
13) *Glycine max*	Custer	0.5	1
14) *Glycine max*	P.I. 79,693	6	3
15) *Glycine max*	P.I. 84,611	58	13
16) *Glycine max*	P.I. 88,788	3	0
17) *Glycine max*	P.I. 209,332	2	0.1
18) *Glycine max*	P.I. 87,631-1	13	10
19) *Glycine max*	P.I. 90,763	8	1
20) *Glycine max*	P.I. 91,684	37	46
21) *Humulus* sp.	Golden Cluster	0	0.1

[a]No cysts were recovered from the following plants also included in the test: *Beta vulgaris, Betula niger, Brassica oleracea capitata* (Flat Dutch Early), Christmas Cactus, *Daucus carota* (Chantenay Red Core), *Ficus* sp., *Nicotiana tabacum* (NC. 95), *Nicotiana tabacum* (VA. 523), *Poa annua, Rumex crispus, Solanum carolinense, Stenotaphrum secundatum, Triticum aestivum* (Benhur), *Trifolium pratense* (Kenland), *T. repens* (Ladino).

[b]Index of parasitism refers to the number of cysts recovered from each test plant expressed as percent of those recovered from Lee soybean.

recent origin, most likely an autotetraploid. It has twice as many chromosomes as the diploid isolate from which it is suspected to have evolved, and has more than 1½ times the amount of DNA per sperm nucleus. It has larger larvae and cysts, as would be expected from a polyploid, but it is very similar to the diploid isolate in morphology and host range. The close similarity in host range in patricular may suggest that the tetraploid isolate has evolved recently and has not been subjected to any significant directional selection on different host plants.

There is no evidence that the tetraploid isolate evolved via a parthenogenetic stage, although that possibility cannot be excluded. There are no parthenogenetic forms of *H. glycines* or of *H. schachtii,* a close relative of *H. glycines.* However, parthenogenesis operates in *H. trifolii,* a closely related species that is assumed to have evolved from an amphimictic ancestor of the *H. glycines* or *H. schachtii* type (10).

The pattern of chromosome pairing in the tetraploid is variable. In some primary oocytes the homologs pair to form 18 bivalents, although they sometimes form quadrivalents or trivalents and univalents. Pairing is such that interpretation of the exact nature of the metaphase I chromosomal configurations is often not possible. In spite of the anomalous pairing during prophase I, migration of the chromosomes to the poles during anaphase I appears to be normal in the majority of the oocytes, and the second maturation division appears to be normal in all oocytes. As a result of this behavior, at least 80% of the mature oocytes have the diploid chromosomal complement (n = 18) and only 20% may have one chromosome less (n = 17) or one chromosome more (n = 19). It is suspected that oocytes with abnormal chromosomal complement do not develop to reproductive adults, but because they represent a small percent in the population the tetraploid isolate maintains itself in culture without

any difficulty. The success of this isolate under field conditions, in competition and possibly intercrossing with the diploid population will be the subject of future studies. Preliminary crosses between the tetraploid and the diploid isolates have been successful and have produced viable triploid progeny under laboratory conditions.

The discovery of a tetraploid *H. glycines*, and its isolation and maintenance under greenhouse conditions, is significant because it provides an experimental tool in the study of polyploidy in amphimictic nematodes. Many amphimictic plant-parasitic nematodes have high haploid chromosome numbers (*Meloidogyne*, n = 18; *Anguina tritici*, n = 19), whereas other close relatives and most free-living nematodes have fewer chromosomes, usually fewer than 9. This fact may indicate that extensive chromosomal changes, possibly including polyploidy, may have played a significant role in evolution of the highly specialized plant-parasitic forms of nematodes. Polyploidy, of course, is frequent in plant-parasitic nematodes that reproduce by mitotic (apomictic) parthenogenesis (7).

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