Relative DNA Content and Chromosomal Relationships of some Meloidogyne, Heterodera, and Meloidodera spp. (Nematoda: Heteroderidae)[1]

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Abstract: The relative DNA content of hypodermal nuclei of preparasitic, 2nd-stage larvae was determined cytophotometrically in 19 populations belonging to 13 species of *Meloidogyne, Heterodera* and *Meloidodera*. In *Meloidogyne hapla, M. arenaria, M. incognita* and *M. javanica*, total DNA content per nucleus is proportional to their chromosome number, indicating that chromosomal forms with high chromosome numbers are truly polyploid. *M. graminicola, M. graminis* and *M. ottersoni* have a DNA content per chromosome significantly lower than that of the other *Meloidogyne* species. Within *Heterodera*, species with high chromosome numbers have proportionally higher DNA content, indicating again polyploidy. DNA content per chromosome in *Meloidogyne* is one third that of *Heterodera* and one half that of *Meloidodera floridensis*. The karyotypic relationships of the three genera are still not clearly understood. *Key Words:* aneuploidy, parthenogenesis, nematodes.

Comparisons of DNA content of related species of animals, particularly of groups with extensive variation in chromosome numbers, have been useful in clarifying karyotypic and phylogenetic relationships (1, 3). The nematode family Heteroderidae is unique with regard to the variety of chromosome numbers encountered in various members (8). Many species of *Heterodera* are diploid with 2n=18 chromosomes, but some appear to be polyploid with somatic chromosome numbers of 24, 26, 27, 32 or 34. Most *Meloidogyne* species, on the other hand, have 2n=36 chromosomes and reproduce by facultative meiotic parthenogenesis or amphimixis, whereas others are mitotic parthenogenetic and have somatic chromosome numbers ranging from 34 to 54. Some nominal species of *Meloidogyne* in fact are known to include more than one chromosomal form. Thus, *M. arenaria* exists as a form with approximately 36 chromosomes and another with 51 to 54 chromosomes. Furthermore, *Meloidodera floridensis*, the only species of this genus studied thus far, is mitotic parthenogenetic with 26 and 27 chromosomes.

Various interpretations of the karyotypic relationships within and between genera of the family Heteroderidae have been expressed (7). It is generally assumed that polyploidy occurs within each genus and is always confined to obligatorily parthenogenetic members. The relationship of the karyotypes of the various genera, however, is more difficult to understand. Since *Meloidogyne* (n=18) has twice as many chromosomes as *Heterodera* (n=9), it is possible that the *Meloidogyne* karyotype represents a tetraploid state of the *Heterodera* karyotype. *Meloidogyne* chromosomes, however, are significantly smaller than *Heterodera* chromosomes. This suggests that *Meloidogyne* karyotype may have been derived from the *Heterodera* karyotype by chromosomal fragmentation or other methods of chromosome number increase, rather than by polyploidization. Alternatively, it can be assumed that *Meloidogyne* karyotype is the ancestral one, and that the *Heterodera* karyotype has evolved from it through centric fusions or other mechanisms of chromosome number reduction.

In view of the uncertainty as to the actual relationships of the various karyotypes within the family *Heteroderidae*, a comparative study of the DNA content of selected members was undertaken. It was hoped that such information would facilitate interpretation of karyotypic relationships within and between genera of this family. A preliminary report of this study has been published (4).

MATERIALS AND METHODS

Freshly hatched preparasitic, 2nd-stage larvae of the populations studied were killed at 65 C, then fixed in 3:1 ethyl alcohol-acetic acid for 4 hr. Following washing in distilled water, larvae were concentrated in 0.5 ml of water in

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No. 3 beam capsules and frozen in dry ice (solid carbon dioxide). They were later sectioned 8 μ thick in a cryostat.

A population of *Meloidogyne incognita* with 2n=42 chromosomes was used as control throughout these studies. Sections were placed in two rows on each slide, with the top row containing the population under study and the bottom row the control for comparison. DNA content was expressed in relative units, with the average measurements of the control from each slide set at 100. All other measurements were adjusted against the control in order to reduce errors due to variation in staining procedures, effects of slides and coverslips, and responses of the photometric equipment.

The material was hydrolyzed in 1 N HCl at 60 C for 10 min, stained in "Schiff reagent modified" (Fisher Scientific Co., Fair Lawn, New Jersey) for 2 hr and mounted in Permount®.

Relative DNA content was determined by the two-wave length method (5, 6) on a Leitz MPV microscope photometer equipped with an interference graded line filter, a Photovolt 520-M photometer, and a low intensity light source with a 6-volt wet cell battery as the power source. Nuclei of the ventral hypodermal chord were chosen for the measurements because they are numerous, compact and uniform in size, easy to identify and show no mitotic activity (Fig. 1).

OBSERVATIONS

Although some variation in chromosome size within the chromosomal complement of each species exists, differences are not very extensive. Therefore, the average DNA content per chromosome of each species is considered in comparison of karyotypes of the various species. On this basis, the various species of *Meloidogyne* can be subdivided into two groups—those with a DNA content per chromosome higher than two, such as *M. hapla, M. arenaria, M. incognita* and *M. javanica*, and those with DNA content lower than two, such as *M. graminicola, M. graminis* and *M. ottersoni* (Table 1).

There is a high positive correlation (r=.992) between the observed and expected DNA content per nucleus within the first group of species (Fig. 2). However, *M. hapla* with 30 chromosomes has considerably higher, and *M. hapla* with 45 chromosomes has considerably lower DNA content than the expected on the basis of their chromosome numbers. Species of the second group fall much below the expected level compared to those of the first group (Fig. 2).

DNA content per nucleus in the diploid species *H. glycines* and *H. schachtii* appears to be somewhat higher than that of *Meloidogyne* species, but DNA content per chromosome is several times higher (Table 1). Since both these diploid species have approximately the same DNA content per nucleus, their average (127.5) was taken as the basis to calculate the expected DNA content of all the other *Heterodera* species. There is a high positive correlation (r=.993) between the observed and expected

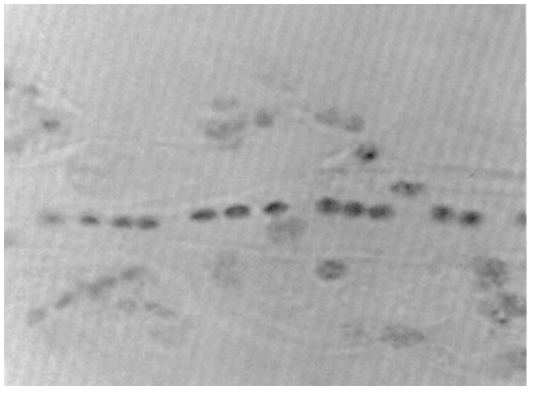

FIG. 1. Photomicrograph of a series of Feulgen-stained, ventral hypodermal chord nuclei of a preparasitic 2nd-stage larva of *Meloidogyne incognita*. 1200 X.

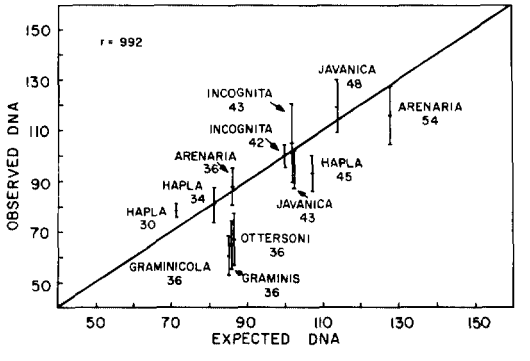

FIG. 2. Relationship between the observed DNA content per nucleus and the amount expected on the basis of chromosome number of various *Meloidogyne* spp. Diagonal line shows DNA values proportional to chromosome numbers, and is based on the DNA value of the control population of *M. incognita* with 42 chromosomes set at 100 (the somatic chromosome number is indicated under each species name. *M. graminicola, M. ottersoni* and *M. graminis* have not been included in calculating the "r" value).

TABLE 1. Relative DNA content of hypodermal nuclei of preparasitic 2nd-stage larvae in various populations and species of *Meloidogyne, Heterodera* and *Meloidodera.*

Species	Chromosome no. n	Chromosome no. 2n	DNA content per nucleus[a]	Average DNA content per chromosome
Meloidogyne				
M. hapla	15	30	76 ± 2.7[b]	2.53
M. hapla	17	34	81 ± 7.1	2.38
M. hapla		45	93 ± 7.1	2.07
M. arenaria		36	88 ± 7.5	2.44
M. arenaria		54	116 ± 11.49	2.15
M. incognita		42	100 ± 4.16	2.38
M. incognita		43	105 ± 15.88	2.44
M. javanica		43	95 ± 7.8	2.21
M. javanica		48	119 ± 10.87	2.48
M. graminicola	18	36	56 ± 7.9	1.56
M. graminis	18	36	65 ± 9.8	1.81
M. ottersoni	18	36	68 ± 10.4	1.89
Heterodera				
H. glycines	9	18	129 ± 12.5	7.17
H. schachtii	9	18	126 ± 8.36	7.00
H. betulae	12	24	196 ± 11.5	8.17
H. sp. from *Rumex* sp.		24	171 ± 10.4	7.13
H. trifolii		26	193 ± 16.9	7.42
H. trifolii		34	202 ± 20.27	5.94
Meloidodera				
M. floridensis		27	113 ± 17.8	4.19

[a]Average of 20 nuclei.
[b]95% confidence limits.

DNA content per nucleus among the various species of *Heterodera* (Fig. 3). However, *H. betulae* with 24 chromosomes and *H. trifolii* with 34 chromosomes, respectively, have a DNA content significantly higher and significantly lower than that which would be expected on the basis of their chromosome number.

Meloidodera floridensis is distinct in that it has a DNA content per chromosome of 4.19 which is significantly different from that of *Meloidogyne* (1.56-2.53) and *Heterodera* (5.94-8.17) species (Table 1).

DISCUSSION

The 1:2 numerical relationship of the basic chromosome numbers of the genera *Heterodera* (n=9) and *Meloidogyne* (n=18) has favored the hypothesis that *Meloidogyne* is a polyploid

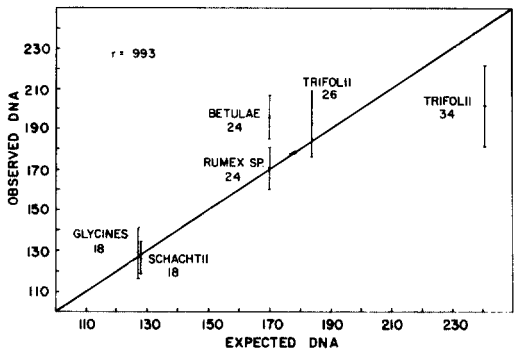

FIG. 3. Relationship between the observed DNA content per nucleus and the amount expected on the basis of chromosome number of various *Heterodera* spp. Diagonal line shows DNA values proportional to chromosome numbers and is based on the average DNA value (127.5) of *H. schachtii* and *H. glycines*, both with 18 chromosomes (the somatic chromosome number is indicated under each species name).

derived from a *Heterodera*-type karyotype. The present study, however, shows that *Meloidogyne* DNA content per chromosome is approximately one third that of *Heterodera*. Therefore, if the above hypothesis is correct, it should be assumed that a significant reduction in the amount of DNA occurred together with, or following, polyploidization. Although this assumption may not be unreasonable, still such a drastic reduction in DNA content per chromosome of *Meloidogyne* may be more easily interpreted as the result of karyotype evolution by chromosomal fragmentation rather than by polyploidization.

Alternatively, it can be assumed that chromosomal evolution has followed the opposite direction, and *Heterodera* karyotype has evolved from *Meloidogyne* karyotype, through centric fusions and other mechanisms of chromosome number reduction. This view is partially supported by the trend for chromosome number reduction that is evident within the genus *Meloidogyne*. Thus, *M. hapla* populations with n=17, 16 or 15 are regarded to have been evolved from other *Meloidogyne* forms with n=18. Still, there are several objections to the hypothesis that *Heterodera* karyotype evolved from the *Meloidogyne* karyotype. Total DNA content per nucleus of *Heterodera* would be expected to be equal or lower rather than higher compared to that of *Meloidogyne*, because of loss of very small chromosomal fragments usually associated with centric fusions. Furthermore, it is peculiar that the chromosome number was reduced to exactly one half the original number (from 18 to 9) and not any other number. Also, it is difficult to assume that the predominantly amphimictic genus *Heterodera* evolved from a predominantly parthenogenetic genus such as *Meloidogyne*.

In general, therefore, the karyotypic relationships of the genera *Heterodera* and *Meloidogyne* are still not understood. The difficulty of establishing a definite relationship between the karyotypes of these two genera may actually indicate the lack of a close relationship between them (7).

Within the genus *Meloidogyne*, the two *M. hapla* populations that undergo meiosis (n=15 and 17) appear to have the same total DNA content. This means that whatever the pathway of derivation of these forms has been, it involved a rearrangement of the same genetic material rather than addition or elimination of chromosomes. The population with 45 chromosomes which undergoes no meiosis, and which has been considered to be a triploid, has slightly more DNA per nucleus but the value is not proportional to its chromosome number. Therefore, if this population is indeed a triploid, some reduction of DNA must have occurred following polyploidization.

In *M. arenaria, M. incognita* and *M. javanica*, total DNA content is proportional to their chromosome number, indicating that chromosomal forms with high chromosome numbers are truly polyploid, or derivatives of polyploids.

M. graminicola, M. graminis and *M. ottersoni*, all with n=18 chromosomes and undergoing meiosis, have a DNA content per chromosome significantly lower than the other *Meloidogyne* species. The two latter species were originally assigned to the genus *Hypsoperine*, which was later synonymized with *Meloidogyne* (9). Golden (2), however, insists that the genus *Hypsoperine* is a valid genus, and the present study adds some evidence in support of his view.

Within the genus *Heterodera, H. glycines* and *H. schachtii* are diploid amphimictic with n=9 chromosomes and have the same DNA content. *H. betulae*, however, is meiotic parthenogenetic with n=12 chromosomes and has proportionally higher DNA content. This indicates that the higher chromosome number of this species has resulted from the addition of chromosomes rather than fragmentation of the chromosomes of the original karyotype. The undescribed species from *Rumex* with 24 chromosomes and one population of *H. trifolii* with 26 chromosomes have DNA content proportional to their chromosome numbers, indicating that they are polyploids or polyploid derivatives. *H. trifolii* with 34 chromosomes has slightly less DNA than expected on the basis of its chromosome number, suggesting that, as in the case of the polyploid *M. hapla*, some reduction in the amount of DNA per chromosome has occurred.

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