DNA Content and Variation in Chromosome Number in Plant Cells Affected by *Meloidogyne incognita* and *M. arenaria*

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ABSTRACT

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DNA content of *Meloidogyne*-induced giant cell nuclei in four host species was determined by Feulgen microspectrophotometry. Nuclei from mature giant cells in pea and tomato averaged 15.8 and 14.2 times more DNA, respectively, than the 2*C* values from uninfected root-tip nuclei. Nuclei from broad bean and lettuce showed a mean increase in DNA content of 8.3- and 6.5-fold, respectively, when compared to the 2*C* values of uninfected root tip nuclei. DNA content of giant cell nuclei was highly variable (coefficients of variation $> 41\%$), while DNA content of root tip cells showed little variation (coefficients of variation $< 8\%$). Mean DNA content in giant cell nuclei from peas increased linearly from the time of inoculation until development of mature female nematodes, 3 wk after inoculation. In giant cells from lettuce, DNA content per nucleus increased 6.5-fold during the first week after inoculation with no further increase later on. Cytogenetic studies of pea giant cells showed that the majority of the nuclei were aneuploid with chromosome counts ranging from 15 ($2n = 14$) to over 100. Also observed were lagging chromosomes and chromatin bridges between giant cell nuclei, which could generate the observed aneuploidy.

When plant-parasitic nematodes of the genus *Meloidogyne* infect a host, they induce the formation of giant cells in the host roots which become the primary feeding site of the nematode. Giant cells are large, densely cytoplasmic, and multinucleated (2). The two to 12 giant cells per feeding site (22) are believed to act as transfer cells, increasing the flow of nutrients from the plant to the nematode (13). The giant cells have a highly elevated metabolism, though it is a generalized increase rather than one of specific metabolic pathways (13). The multinucleated state, long a source of debate (3,4,10,20,22), is believed to arise from repeated mitosis of a single cell without cytokinesis (14). The mechanism by which the nematodes induce these host cell modifications is unknown but is believed to involve secretions from esophageal gland cells (12,23).

The chromosomal state of the giant cell nuclei is not understood. The DNA content of these nuclei is known to be variable, but

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little specific data are available (14). Earlier cytogenetic studies have revealed exclusively euploid nuclei but with different numerical progressions of chromosomes per nucleus observed. Huang and Maggenti (10) examined over 100 giant cells induced by *Meloidogyne javanica* (Treub) Chitwood from *Vicia faba* L., arrested in metaphase or anaphase, and subjected them to chromosome number determination, either by direct count or by M chromosome ratio (M chromosomes are large, metacentric chromosomes with distinct satellites and are present in one pair per diploid genome). All of the giant cell nuclei contained a euploid number of chromosomes and fit a strict geometric progression ($2n$, $4n$, $8n$, etc.). Bird (4), however, also working with *M. javanica* and *V. faba*, observed different chromosome patterns. The nuclei were euploid, but no clear progression to the counts was observed. The possibility of aneuploid nuclei has been suggested but with no supporting cytological data (4,14).

The objectives of this study were to determine the amount of DNA per giant cell nucleus and the extent of DNA content variability among giant cell nuclei, to quantify changes in DNA content with time, and to determine whether aneuploidy is involved in the abnormal nuclear development of giant cells.

MATERIALS AND METHODS

DNA content determinations of mature giant cell nuclei were made for four host plant species: broad bean (*Vicia faba*), pea (*Pisum sativum* L. 'Little Marvel'), lettuce (*Lactuca sativa* L. 'Grand Rapids'), and tomato (*Lycopersicon esculentum* Mill. 'Rutgers'). All were grown in a sand/peat mix (tomatoes) or in rag dolls (broad beans, peas, and lettuce) as described by Carter et al (6). Seedlings in rag dolls were grown at a 24 C day/20 C night temperature cycle and a 12-hr light/dark cycle. They were inoculated at 6 days of age with 75–100 juveniles of *Meloidogyne incognita* (Kofoid & White) Chitwood per seedling. Tomatoes in the sand/peat mix were grown in a greenhouse (22–30 C) and inoculated with approximately 5,000 eggs of *M. arenaria* (Neal) Chitwood per seedling.

In both systems, plants were allowed to grow until mature giant cells (denoted by the presence of nematode egg masses on the exterior of the gall) were present at 4–6 wk after inoculation. Once mature giant cells were present, infected root sections were cut from the plant and fixed in 3:1 (v/v) absolute ethanol/glacial acetic acid for 24 hr. After fixing, material could be stored in 70% ethanol for up to several months without tissue breakdown.

Feulgen staining was conducted via the protocol used by Price et al (21). Fixed specimens were hydrolized in 5 N HCl at 25 C for 40 min and then placed in Schiff's reagent for 2 hr. Schiff's reagent was prepared by dissolving 2 g of basic fuscin (Fisher certified biological stain) in 400 ml of boiling water and, after cooling, adding 4 g of potassium metabisulfite ($K_2S_2O_5$) and 40 ml of 1 N HCl. This was stored overnight in a dark bottle. The next day, before use, 1 g of Norit A decolorizing charcoal was added and the mixture was shaken and then vacuum-filtered through Whatman No. 1 filter paper, producing a clear stain. Stained material was rinsed twice in SO_2 water (600 ml of H_2O, 36 ml of 1% $K_2S_2O_5$, and 30 ml of 1 N HCl) and once in distilled water for 10 min each.

Stained samples were then examined with a stereo microscope and giant cells teased out of the galls, placed on a microscope slide in a drop of 45% acetic acid, and squashed under a coverslip. The slides were rapidly frozen in liquid nitrogen and the coverslips were popped off with a scalpel blade. After drying overnight, permanent coverslips were applied over Permount (Fisher).

Mature giant cell nuclei and similarly prepared root-tip nuclei were subjected to microspectrophotometry to determine relative DNA contents (expressed as Feulgen absorbance units: FAU). All root-tip nuclei scanned were in mid-prophase and thus contained the $4C$ amount of DNA; the $2C$ DNA content was obtained by halving these values. The apparatus was a Zeiss Universal-II scanning microspectrophotometer with a 0.5-μm scanning stage connected to a Zonax computer with Zeiss Bioscan software. Individual nuclei were scanned under oil immersion, at 560 nm, and at a magnification of ×100/1.25. Spectrophotometric measurements were made on several giant cell nuclei per gall from a minimum of four galls from separate plants of each host species.

Time course studies were performed on peas and lettuce to determine the rate of DNA content increase in the giant cells. These plants were grown and treated as described above except that infected root sections were taken and fixed weekly from inoculation until mature giant cells were found at 4 wk after inoculation.

Cytogenetic studies were conducted on peas only. The plants were grown and inoculated in rag dolls. Infected root sections were taken every day from 6 to 18 days after inoculation to determine the optimum time for obtaining mitotic figures. They were then incubated in 0.1% colchicine for 8 hr to arrest the giant cell nuclei at metaphase. After incubation, root sections were fixed and stained, and slides of the giant cell nuclei were made as described earlier. These nuclei were examined at ×1000 magnification and chromosomes were counted.

Mitotic figures of giant cell nuclei from pea were also obtained without the use of colchicine. Infected root sections were taken at 8 days after inoculation (determined to be the optimum age for obtaining mitotic figures) at 1-hr intervals from 8 to 11 a.m. and fixed immediately. They were stained and prepared as squashes as before. In an attempt to localize the nucleolar organizer region, silver staining (19) on both Feulgen- and acetocarmine-stained metaphase squashes and Fast Green staining on Feulgen-stained material (11) was performed.

RESULTS

Mature giant cell nuclei from all host species had increased DNA contents as compared to $2n/2C$ root-tip cell nuclei (Table 1). The DNA content of giant cell nuclei was highly variable with coefficients of variation of 41 to 58% as compared to root-tip nuclei coefficients of 5 to 8%.

Time course studies revealed that although DNA content of individual nuclei was highly variable, the increase in mean DNA content per nucleus in pea giant cells from the time of inoculation until the appearance of mature female nematodes at 3 wk post-inoculation was linear (Fig. 1). The mean DNA content per nucleus was little changed from week 3 to week 4. In lettuce, however, the giant cell nuclei had reached maximum mean DNA content by 1 wk after inoculation; from week 1 to week 3, the mean DNA per nucleus content remained unchanged (Fig. 1).

Chromosomes could be counted clearly in 31 giant cell nuclei from galls from several plants at 8 days postinoculation. The majority of these nuclei were aneuploid and the counts followed no discernible pattern (Fig. 2). Nuclei with chromosome counts ranging from 17 ($2n$ = 14) to 88 (Fig. 3) were observed. For many nuclei, there were too many chromosomes to accurately count but best estimates were that each of these nuclei had over 100 chromosomes. The mitotic figures of giant cells prepared without colchicine provided evidence of mitotic abnormalities. These ranged from persistent chromatin bridges to bilobed interphase nuclei (Fig. 4). During the course of the cytogenetic

TABLE 1. Relative DNA contents of root-tip and nematode-induced giant cell nuclei

Host[a]	Root tip cells		Giant cells		Fold increase
	FAU[b]	CV[c]	FAU[b]	CV[c]	
Lycopersicon esculentum	17.9	8%	255.6	47%	14.2
Vicia faba	334.1	5%	2,779.8	58%	8.3
Pisum sativum	81.8	5%	1,289.6	41%	15.8
Lactuca sativa	38.9	5%	254.0	55%	6.5

[a] All plants infected with *Meloidogyne incognita* except *L. esculentum*, which was infected with *M. arenaria*. Each value represents the mean of at least 16 measurements from four different plants.
[b] FAU = Feulgen absorbance units.
[c] CV = coefficient of variation.

studies, it was noted that many of the giant cell nuclei contained small pieces of Feulgen-positive material which appeared near the ends of some chromosomes (Fig. 3). Silver staining was performed on both the old Feulgen-stained slides and newly prepared squashes but was inconclusive. Fast Green stain also failed to reveal any connections between chromosomes and the small pieces of material.

DISCUSSION

The DNA content of mature giant cell nuclei was found to be both highly elevated and highly variable; individual nuclei ranged from just above the 2*C* root tip DNA level to over 20 times the 2*C* level. Additionally, the DNA contents showed no geometric increase that would be expected from nuclear formation by endoreduplication or endopolyploidy. This implies that the DNA increase, on a per nuclear basis, is not a systematic increase of all the genomic material. It has been suggested that this increase is caused by specific gene or sequence amplification (14) but with no supporting data.

Further evidence for a nonsystematic giant cell DNA increase comes from the cytogenetic studies conducted on peas. Metaphase figures were lacking in giant cells from pea older than 12 days after inoculation, whereas DNA content continued to increase until 21 days after inoculation. This implies a method of DNA increase other than endomitosis. Possibilities include polyteny (endoreduplication), heterochromatin addition, and amplification of selected DNA sequences. A/T rich satellite DNA has been found to be amplified in the protocorms of the orchid *Cymbidium*

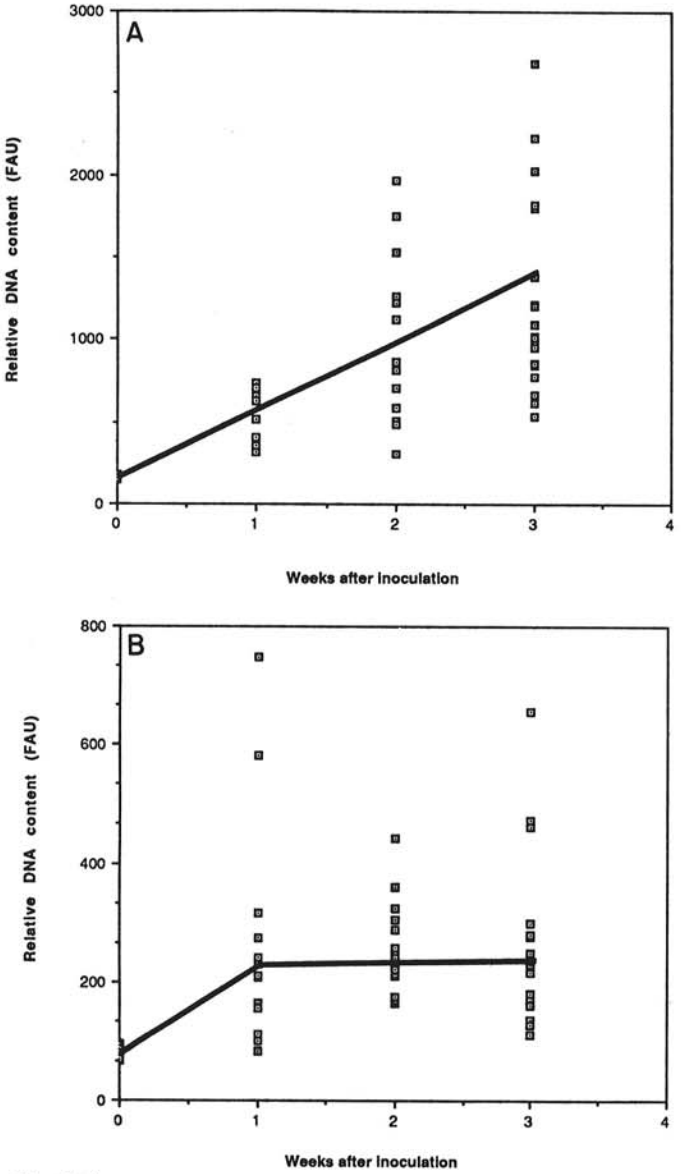

Fig. 1. Time course analysis of the increase in DNA per nucleus in giant cells from **A,** pea and **B,** lettuce. There were a minimum of 12 observations per host species each week. Solid line represents rate of change of mean values of DNA per nucleus. FAU = Fuelgen absorbance units.

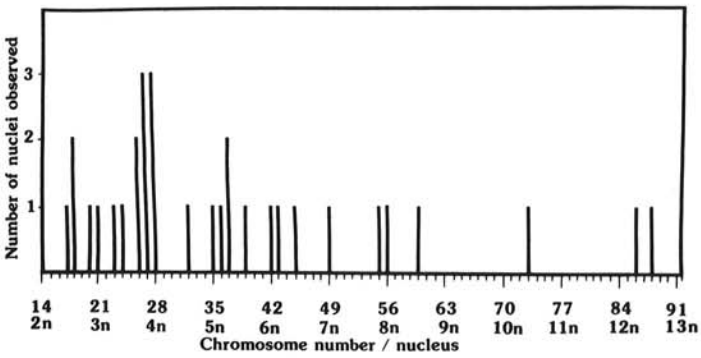

Fig. 2. Graphic representation of giant cell chromosome counts on a per nucleus basis. Counts were obtained from giant cells in pea at 8 days after inoculation and come from individual galls on six separate plants.

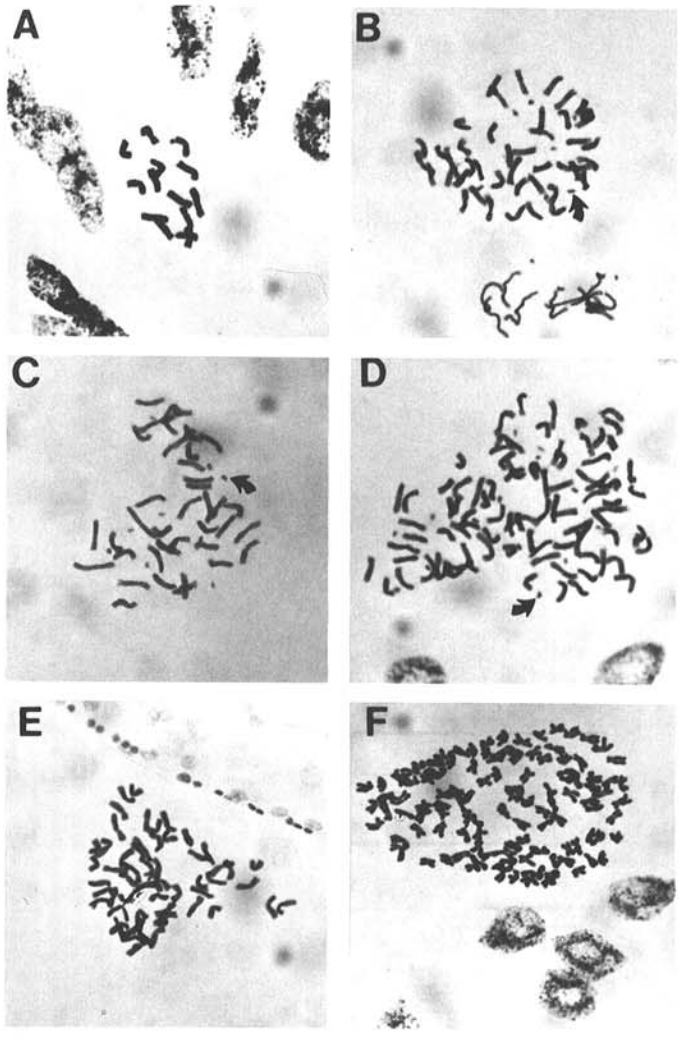

Fig. 3. Mitotic spreads of nuclei from giant cells in pea. Chromosome numbers are as follows: **A,** $2n = 14$; **B,** 45; **C,** 37; **D,** 88; **E,** 55; and **F,** approximately 112. Spreads **E** and **F** were obtained without colchieine and lack the Feulgen positive bodies seen the other spreads (indicated by arrows).

Sw. (5) and is manifested as an unequal heterochromatin increase (16). Polyteny has been found in nuclei of the embryonic suspensor organ in *Tropaeolum majus* L. (17). While the linearity of the DNA increase argues against a true endoreduplication (it is linear rather than exponential), it is possible that some sequences are omitted or under-replicated during the process. Under-replication of genomic sequences has been found in differentiating *V. faba* root cells (1); as differentiation progressed, there was a loss of repeated sequence DNA and a total elimination of satellite DNA. Sequence amplification in plants subjected to environmental stress has also been documented. Heritable changes in the copy number of highly repeated sequence families could be experimentally induced in both whole plants (9) and tissue culture (8).

Cytogenetic studies further revealed the prevalence of aneuploid nuclei within giant cells. In contrast to previous studies (4,10), we found most of the giant cell nuclei to be aneuploid with a large range and no pattern of chromosome counts. It is possible that aneuploidy may result from an unequal distribution of genetic material during mitosis and/or from retention of only part of the chromosomal complement; such a phenomenon occurs in sunflower (7,15).

The observed aneuploidy prompted a search for mitotic aberrations that might result in such a condition. Nuclei with chromatin bridges were found, indicating an abnormal division of genetic material. Some telophase nuclei had large chromatin bridges and appeared fused, a theory supported by the observation of bilobed interphase nuclei (Fig. 4). It appears, then, that aneuploidy may be generated by an abnormal division of genetic material while some higher ploidy levels may be reached by nuclear fusion. Huang and Maggenti (10) noted similar chromatin bridges buy did not speculate on their role in giant cell nuclear formation.

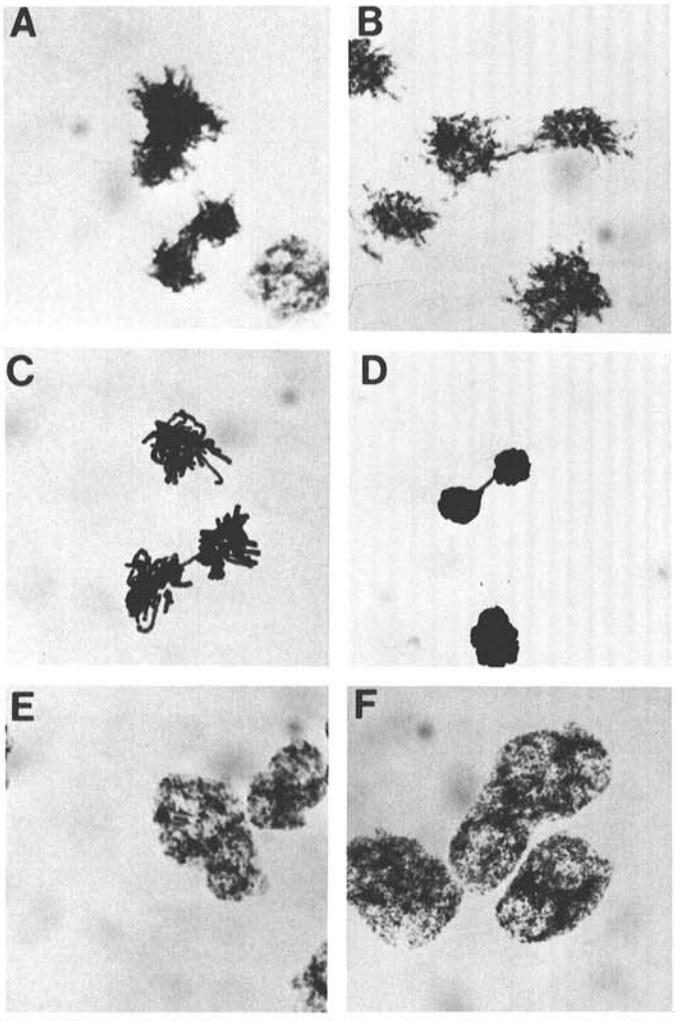

Fig. 4. Giant cell mitotic spreads with chromatin bridges. In **A**, the bridges are such that nuclear fusion appears to be occurring. A more tenuous bridge, from which the chromatin may be lost, exists in **B**. **C** and **D** show metaphase and telophase squashes, respectively, with lagging chromosome bridges. **E** and **F** show bi-lobed interphase nuclei possibly resulting from the type of nuclear fusion depicted in **A**.

As previously discussed, no metaphase spreads were found in giant cells from pea beyond 12 days after inoculation, implying that a mitotic stimulus present in young cells is absent in older cells. This is in agreement with Bird (4), who found that mitosis was absent in giant cells associated with postmolt nematodes.

Time course studies revealed that giant cell nuclear development may proceed differently in different hosts. Even though the nematode developed at the same rate in peas and lettuce (mature females were present in both at 3 wk after inoculation and egg production was noted at 4 wk), the pattern of giant cell DNA increase was different between the two hosts (Fig. 1). The stabilization in DNA content from week 3 to 4 in pea is not unexpected. The rate of giant cell development is believed to be correlated with that of the nematode (20); thus, when the nematode reaches maturity, the giant cell does also. The much earlier stabilization of DNA content in lettuce (after 1 wk) was surprising and may be related to the fact that members of the Asteracea do not normally undergo any kind of endopolyploidy (18). Thus, formation of the giant cell nuclei may be hampered and the increase in DNA terminates much earlier than in pea.

This paper presents, for the first time, data that quantify the changes in DNA content during giant cell nuclear development. Cytological observations also revealed for the first time the presence of aneuploid nuclei in giant cells. Mechanisms were observed that could generate the observed aneuploidy. DNA content appears to increase in linear fashion, suggesting a nonsystematic increase, on a per nucleus basis, of the genomic material. The nature of this nonsystematic increase is not known at this time.

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