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DNA Complexity of the Root-knot Nematode (*Meloidogyne* spp.) Genome[1]

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Abstract: Cot curves derived from renaturation kinetics of sheared denatured DNA indicated that the genome of six populations representing the four most common root-knot nematode species (*Meloidogyne incognita, M. arenaria, M. javanica,* and *M. hapla*) is composed of 20% repetitive and 80% nonrepetitive sequences of DNA. Cot curves were almost identical, indicating that all populations had a haploid genome of approximately the same size. Calculations from an average Cot curve gave an estimate of 0.51×10^8 nucleotide base pairs for the haploid genome of the four *Meloidogyne* species. This genome is about 12–13 times larger than the genome of the *E. coli* strain used as a control.

Key words: Cot analysis, DNA, *Meloidogyne,* repetitive DNA, root-knot nematode, unique DNA.

The kinetics of renaturation (formation of duplex DNA) of the complementary strands of DNA can supply basic information about the sequence organization of the genome and its complexity (2,13). It has provided evidence of the presence of repetitive sequences and estimates of degree of repetition, as well as the length of the repeated sequences in eukaryotic DNA (2). Renaturation of sheared total eukaryotic DNA also has revealed the presence of another class of DNA composed of unique or nonrepetitive sequences interspersed with repetitive sequences (11). The DNA of the free-living nematode *Caenorhabditis elegans* was found to consist mostly of nonrepetitive sequences and a relatively small fraction of repetitive sequences interspersed throughout the entire genome, as in other eukaryotes (4,10). On the other hand, characterization of the genome of the free-living nematode *Panagrellus silusiae* revealed that the nonrepetitive DNA was only infrequently interspersed with repetitive sequences (1).

This paper reports findings about the complexity of the genome of four species of root-knot nematodes (*Meloidogyne* spp.) determined by rates of renaturation of sheared denatured native DNA. Complexity here refers to the presence of different classes of DNA sequences, their length expressed in number of nucleotide base pairs, and the size of the haploid genome. Organization of the different classes of DNA sequences is not included.

Materials and Methods

Six populations representing various cytological and reproductive forms of the four most common root-knot nematode species, i.e., *Meloidogyne incognita* (race A), *M. javanica, M. arenaria* (one diploid and one triploid), and *M. hapla* (one meiotic and one mitotic) (12), were investigated. Propagation and maintenance of each population, obtaining nematode materials for DNA extraction, and DNA isolation by cesium trifluoroacetate (CsTFA) ultracentrifugation were conducted as previously described (7).

Shearing and sizing of DNA fragments: DNA preparations in 0.12 M sodium phosphate–0.001 M EDTA were sheared by two passages through an Aminco French Pressure cell at 20,000 psi at a rate of 30–35 drops per minute. The size of the fragments was estimated by horizontal electrophoresis in 1.2% agarose gels using lambda DNA digested with Hind III restriction enzyme as a molecular weight marker. The gels were electrophoresed at 30 V for 20 hours in 90 mM Tris-borate buffer (90 mM

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Trizma-base, 90 mM boric acid, 1.8 mM disodium ethylenediaminetetraacetic acid [Na_2EDTA], pH 8).

DNA renaturation: Parafilm-sealed screw-cap tubes containing 40–500 μg of sheared, heat-denatured DNA per milliliter of 0.12 M sodium phosphate buffer were incubated for different lengths of time at 60 C to allow renaturation. Time periods selected were based on the initial concentration of DNA and estimated Cot values (moles of nucleotides of the initial concentration of denatured DNA times the length of incubation in seconds per liter). Renaturation kinetics were determined by the following procedure:

Renatured double-stranded and unrenatured single-stranded DNA were separated through a water-jacketed hydroxyapatite column or through a prepacked "mini-resin column" (Bethesda Research Laboratories) attached to a 3-ml disposable syringe. When a hydroxyapatite column was used, unrenatured DNA was eluted five times with 4 ml of 0.12 M sodium phosphate at 60 C. The temperature was then raised to 98–100 C to elute the renatured DNA as single-stranded DNA, with the same procedure employed earlier for the elution of the unrenatured DNA (1). When the mini-resin column was employed, the renaturation reactions were diluted 10-fold with Buffer C (0.5 M NaCl in 20 mM Tris-HCl, pH 7.2, 1 mM EDTA), which acted as the binding buffer for the single-stranded, unrenatured DNA sequences and as the elution buffer for the double-stranded, renatured sequences. Five milliliters of Buffer D (2.0 M NaCl in 20 mM Tris-HCl, pH 7.2, 1 mM EDTA) were used later to elute the single-stranded unrenatured DNA sequences from the mini-column which was attached to a 3-ml disposable syringe. The renatured, double-stranded DNA was denatured by immersion in water at 98–100 C for 4–5 minutes. The concentrations of renatured and unrenatured DNA were determined in a spectrophotometer at 260 nm wavelength, and the fraction of renatured DNA was estimated from these concentrations. DNA of *E. coli* strain K12 was used as a reference in this experiment (3). The observed $Cot_{½}$ values were subsequently corrected for the effect of the presence of repetitive sequences on the rate of renaturation of the nonrepetitive sequences, as recommended by Laird and McCarthy (5).

Results

DNA isolation and size of DNA fragments: The DNA isolated by the CsTFA ultracentrifugation method did not need further purification for renaturation reactions (7). Agarose gel fractionation with Hind III digested lambda DNA used as a molecular weight marker indicated that the mean size of the fragments of *Meloidogyne* DNA was approximately 550 nucleotides.

DNA renaturation: The rate of renaturation of sheared DNA, 550 nucleotide fragments long, is illustrated in Figure 1 as a Cot curve where the fraction renatured (1 – C/Co) is plotted against the log of Cot. Hydroxyapatite and the mini-resin column binding measurements showed that there are two classes of DNA sequences in the root-knot nematode genome. One class is composed of rapidly renaturating sequences, reassociating before a Cot of 1, and constitutes 20% of the genome (corresponds to 0.20 fraction renatured along the Y axis). The other class includes slowly renaturating sequences that constitute 80% of the genome (correspond to 1 − 0.20 = 0.80 fraction renatured). Negligible, if any, reassociation occurred at the next log of Cot. This finding implies that there is a clear separation between DNA sequences that renature rapidly and those that renature slowly. The slowly renaturating fraction requires a $Cot_{½}$ of 2×10^2 (mole)(sec)/liter at half renaturation, whereas the rapidly renaturing fraction requires a $Cot_{½}$ of 2.8×10^{-3} (mole)(sec)/liter. The presence of 20% of rapidly renaturing DNA at the start of renaturation (Co) slows down the rate of renaturation of the slowly renaturing DNA by a factor of 0.2. Therefore, a corrected, true $Cot_{½}$ value for the slowly renaturing sequences is calculated as 1.60×10^2 (mole)(sec)/liter.

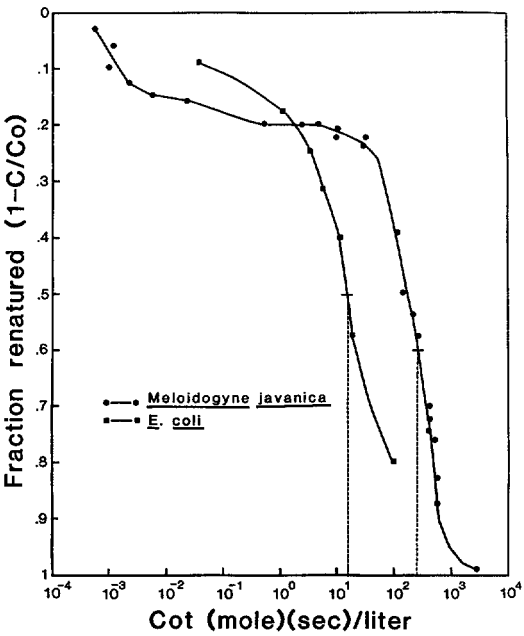

FIG. 1. Renaturation of *Meloidogyne javanica* DNA measured by hydroxyapatite and mini-resin column chromatography. *E. coli* DNA is shown for comparison. Co and C are the concentrations of denatured, single-stranded DNA at zero time and time t in seconds, respectively. Rapid and slow renaturating components comprise 20% and 80% of the genome, respectively. Almost identical curves were generated for the other five populations of *Meloidogyne* studied.

The observed experimental rates of renaturation, as calculated from the formula $k = 1/Cot_{½}$ (2,5,13) are 3.6×10^{2} liter/(mole)(sec) for the repetitive, and 5×10^{-3} liter/(mole)(sec) for the nonrepetitive DNA fractions. The rate of renaturation was quite similar for all *Meloidogyne* species, since the points generated from several series of varying concentrations from all populations fitted into the single curve generated for *M. javanica* (Fig. 1).

Using strain K12 of *E. coli* DNA as reference, the size of the slowly renaturing component of the *Meloidogyne* DNA was estimated as 0.41×10^{8} base pairs (approximately 2.7×10^{10} daltons) per genome. This estimate was calculated from the corrected $Cot_{½}$ value (1.60×10^{2}) of the slowly renaturing fraction of *Meloidogyne* on the basis that genome lengths of *Meloidogyne* (?) and *E. coli* (4.2×10^{6}) are proportional to their $Cot_{½}$ values. Calculations provided an estimate of 0.1×10^{8} base pairs (approximately 6.6×10^{9} daltons) for the rapidly renaturing fraction of *Meloidogyne* DNA (20% of the total) and 0.51×10^{8} base pairs (approximately 3.36×10^{10} daltons) for the entire haploid genome.

DISCUSSION

Cot curves generated by these renaturation kinetics studies for all six nematode populations were almost identical. Such results suggest a very close similarity in the relative amount of repetitive and unique DNA sequences, as well as in the size of the haploid genome of the various root-knot nematodes. The estimated genome size of 0.51×10^{8} base pairs for *Meloidogyne* spp. is approximately 12–13 times larger than the genome size of *E. coli*. Small haploid genome sizes have been estimated also for *Caenorhabditis elegans* (0.8×10^{8} base pairs) (10) and *Panagrellus silusiae* or *P. redivivus* (0.9×10^{8} base pairs) (1,8), whereas slightly larger sizes have been reported for the animal-parasitic nematodes *Ascaris suum* and *Trichinella spiralis* (9).

Earlier investigations had demonstrated that the amount of DNA per nucleus in *M. incognita* (2n = 36–46), *M. arenaria* (2n = 36–54), *M. javanica* (2n = 43–48), and *M. hapla* (2n = 30–45) is approximately proportional to their chromosome numbers (6). The present study suggests that the populations studied have the same haploid genome size and that their genome contains similar amounts of repetitive and nonrepetitive DNA sequences. The fact that the various nematode populations had different numbers of chromosomes does not influence interpretation of the results of the present study. A polyploid population is expected to have more copies of its haploid genome per nucleus than a diploid population. However, renaturation reaction solutions of the same amount of DNA should contain the same number of copies of haploid genome, independent of the degree of ploidy of the populations, if haploid genome size is the same in all populations. Otherwise, the number of copies of haploid genomes should be inversely propor-

tional to genome size of the populations. For this reason, the degree of ploidy does not affect the rate of renaturation or the shape of the Cot curves in our tests. Actually, it is this property of reassociation kinetics that permits determination of the size of the genome in terms of number of base pairs and units of molecular weight and thus allows comparisons of the DNA of distantly related organisms, such as *E. coli* and nematodes.

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