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Unusual and Strongly Structured Sequence Variation in a Complex Satellite DNA Family from the Nematode *Meloidogyne chitwoodi*

Philippe Castagnone-Sereno,[1] Hélène Leroy,[1] Jean-Philippe Semblat,[1] Frédéric Leroy,[1] Pierre Abad,[1] Carolien Zijlstra[2]

[1] INRA, Laboratoire de Biologie des Invertébrés, BP 2078, 06606 Antibes cedex, France
[2] IPO-DLO, Research Institute for Plant Protection, PO Box 9060, NL 6700 GW, Wageningen, The Netherlands

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Abstract. An *Alu*I satellite DNA family has been isolated in the genome of the root-knot nematode *Meloidogyne chitwoodi.* This repeated sequence was shown to be present at approximately 11,400 copies per haploid genome, and represents about 3.5% of the total genomic DNA. Nineteen monomers were cloned and sequenced. Their length ranged from 142 to 180 bp, and their A + T content was high (from 65.7 to 79.1%), with frequent runs of As and Ts. An unexpected heterogeneity in primary structure was observed between monomers, and multiple alignment analysis showed that the 19 repeats could be unambiguously clustered in six subfamilies. A consensus sequence has been deduced for each subfamily, within which the number of positions conserved is very high, ranging from 86.7% to 98.6%. Even though blocks of conserved regions could be observed, multiple alignment of the six consensus sequences did not enable the establishment of a general unambiguous consensus sequence. Screening of the six consensus sequences for evidence of internal repeated subunits revealed a 6-bp motif (AAATTT), present in both direct and inverted orientation. This motif was found up to nine times in the consensus sequences, also with the occurrence of degenerated subrepeats. Along with the meiotic parthenogenetic mode of reproduction of this nematode, such structural features may argue for the evolution of this satellite DNA family either (1) from a common ancestral sequence by amplification followed by mechanisms of sequence divergence, or (2) through independent mutations of the ancestral sequence in isolated amphimictic nematode populations and subsequent hybridization events. Overall, our results suggest the ancient origin of this satellite DNA family, and may reflect for *M. chitwoodi* a phylogenetic position close to the ancestral amphimictic forms of root-knot nematodes.

Key words: Evolution — *Meloidogyne chitwoodi* — Parthenogenesis — Satellite DNA — Sequence variability

Introduction

Repetitive DNA represents a large proportion of most eucaryotic genomes. Tandemly arranged, highly reiterated sequences, known as satellite DNAs, are generally concentrated in centromeric and telomeric regions of a chromosome and constitute the principal component of heterochromatin (Miklos 1985). The length of the repeat units ranges from 2 to 2,000 bp (Miklos and Gill 1982), and their abundance can vary from less than 1% to more than 66% of the genome (Skinner 1977). Despite extensive attempts to assign a role to these noncoding sequences, their biological function has not yet been established. In fact, the great sequence similarity usually observed among all copies of a satellite DNA family is in contrast with the hypothesis that they may not be influ-

Correspondence to: P. Castagnone-Sereno; e-mail pca@antibes.inra.fr
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enced by sequence-specific selection pressure mechanisms. This was accounted for by the theory of molecular drive and concerted evolution, assuming that the nucleotide sequences of the multiple copies of a satellite DNA family are homogenized by recombination processes such as unequal crossing-over and/or gene conversion within a common gene pool (Dover 1982a). It has also been proposed that the putative function(s) and turnover of satellite DNA could affect both the genome structure and the evolution of the organism (Rose and Doolittle 1983). From that point of view, the description of the structural features of satellite DNA is of importance to provide insight into genome dynamics (Dover 1982b).

Root-knot nematodes of the genus *Meloidogyne* constitute the most widely distributed group of plant-parasitic nematodes. The most frequently encountered species, *M. arenaria, M. incognita,* and *M. javanica,* reproduce exclusively by mitotic parthenogenesis, whereas the remaining 'minor' species (with respect to both their distribution and economic importance on a worldwide basis) can reproduce by either (meiotic or mitotic) parthenogenesis or amphimixis (Triantaphyllou 1979). It is suspected that all the mitotic parthenogenetic forms probably have evolved from meiotic parthenogenetic ancestors, or less likely, from amphimictic ones, following suppression of the meiotic process during maturation of the oocytes (Tryantaphyllou 1985). Based on cytogenetic information, it has also been assumed that the meiotic parthenogenetic forms with reduced chromosome numbers (such as some isolates of *M. hapla* or *M. chitwoodi*) may hold a position closer to the ancestral forms of root-knot nematodes, and may have played a significant role in the evolution of the whole genus (Triantaphyllou 1985). More recently, the study of the organization of their genome has appeared as one possible way to further improve our knowledge of the evolutionary pathway of these nematodes. We have previously isolated and characterized a satellite DNA family from a meiotic parthenogenetic *M. hapla* population, which consists of 169-bp *Sty*I repeated elements (Piotte et al. 1994). As reported for satellite DNA families from many other organisms, a great homogeneity among monomers was observed in this nematode with respect to nucleotide sequence, and the presence of an internal subrepeating unit suggested that this satellite DNA could have evolved from a shorter ancestral sequence. Moreover, the comparative analysis of various other mitotic and meiotic *Meloidogyne* species clearly demonstrated that the distribution of this element is restricted to *M. hapla* isolates only (Castagnone-Sereno et al. 1995; Piotte et al. 1995). Given the polyploid nature of many of the *Meloidogyne* spp. genomes (Triantaphyllou 1985), satellite DNA evolution in this group may have an added layer of complexity as compared with diploid genomes.

In order to gain further insight into evolutionary trends in root-knot nematodes, this paper reports the isolation and characterization of a novel satellite DNA family in the genome of *M. chitwoodi.* In contrast to other nematode satellites, this DNA family shows a sequence variability that is highly structured, with the occurrence of six well-defined subfamilies from a sample of 19 sequenced monomers. Similarities in the monomer length, the A + T content, the nucleotide sequence, and the presence of specific restriction sites have been analyzed both between and within subfamilies in order to study mutational and evolutionary processes acting on these sequences. Such data could give us some information concerning the origin and evolution of satellite DNA within this root-knot nematode species.

Materials and Methods

Nematodes. A *M. chiwoodi* isolate (code Co) originately collected from Horst, The Netherlands was chosen for this study. Cytological studies indicated that this isolate is diploid ($N = 16$) and reproduces by meiotic parthenogenesis (van der Beek, pers. comm.).

Purification of Genomic DNA. Eggs were extracted from infested roots, collected (Hussey and Barker 1973), and placed on a 10-μm pore sieve at 20°C to allow the eggs to hatch for 3 weeks. *Meloidogyne chitwoodi* second-stage juveniles were collected from infested tomato roots, concentrated by centrifugation at 2,000 *g* for 2 min in a 30% sucrose solution, washed in distilled water, and pelleted in a microcentrifuge. The pellet was transferred to a mortar, frozen by liquid nitrogen, and ground. From the resulting fine powder, total DNA was extracted as described previously (Zijlstra et al. 1995).

DNA Analysis. Standard procedures were used for restriction endonuclease digestion, electrophoresis, transfer to nitrocellulose or nylon membranes, radioactive labelling, and hybridization (Sambrook et al. 1989). Hybridizations were performed overnight at 65°C. Conditions for washing consisted of 65°C in a 1 × SSC, 0.1% SDS final solution.

Isolation and Cloning of Satellite DNA. Genomic DNA of *M. chitwoodi* was digested with a set of restriction endonucleases, separated on a 1% agarose gel, and stained with ethidium bromide. To ensure complete digestion, incubations were performed for 4 h at 37°C with 10 units of enzyme/μg of DNA. Putative satellite-DNA restriction fragments were recovered from the gel according to Dretzen et al. (1981), ligated into the plasmid vector pUC19, and used to transform competent *Escherichia coli* DH1 cells according to standard procedures (Sambrook et al. 1989). The transformants were selected on ampicillin (100 μg/ml) agar plates containing X-gal (80 μg/ml) and IPTG (120 μg/ml), and screened by colony hybridization (Grunstein and Hogness 1975) using the DNA fragments isolated from the gel as probes.

Nucleotide Sequencing and Computer Analysis. The nucleotide sequence of inserts from recombinant clones selected at random and corresponding to putative monomers were determined by the dideoxy chain-termination method (Sanger et al. 1977) using double-stranded plasmid as template and a Sequenase kit (U.S. Biochemical Corp.). Multiple sequence alignments and tree construction were performed using CLUSTAL W (Thompson et al. 1994). DNA sequence data were compared with the EMBL and GenBank databases by using the NCBI BLAST server (Altschul et al. 1990).

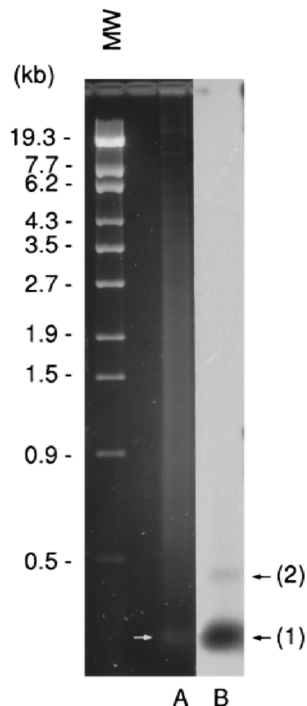

Fig. 1. Restriction analysis of *Meloidogyne chitwoodi* genomic DNA after digestion with *Alu*I. **A** Ethidium bromide stained 1% agarose gel. The white arrow indicates the position of the *Alu*I monomer repeat. **B** Southern blot after hybridization with a mixture of *Alu*I repeat fragments of about 160-bp isolated from the agarose gel. The black arrows (1, 2) indicate the position of the *Alu*I monomers and dimers, respectively.

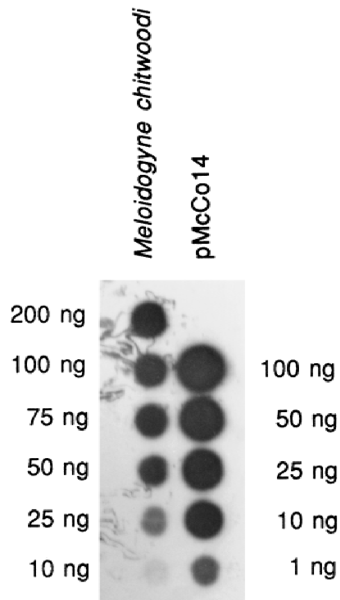

Fig. 2. Abundance of the *Alu*I satellite DNA in *Meloidogyne chitwoodi* genomic DNA determined from dot-blot hybridization. The dot strips containing increasing amounts of *Alu*I cloned monomer and genomic DNA were hybridized with the corresponding monomer (pMcCo14).

Results

Identification and Cloning of a Satellite DNA Family from Meloidogyne chitwoodi

Digestion of *M. chitwoodi* genomic DNA and subsequent electrophoresis revealed the presence of a strong band of approximately 160 bp in *Alu*I digests (Fig. 1A). The *Alu*I band was purified from the gel, ^{32}P-labelled, and used as a probe in Southern blot analysis. The autoradiograph revealed the presence of prominent 160-bp repeats in the *Alu*I digest (Fig. 1B). The *Alu*I pattern also showed that part of the repeated DNA remained as dimers of the 160-bp repeat unit, suggesting tandem repetition of the monomer and loss of the *Alu*I site in some of the units. The arrangement in tandem arrays of this repeated element was confirmed after time-course digestion of *M. chitwoodi* DNA with *Alu*I, which produced a typical ladder pattern after electrophoresis and hybridization with the probe described above (data not shown).

The 160-bp *Alu*I fragments isolated from the gel were subcloned into pUC19 vector. Nineteen positive clones, named pMcCo*n,* were selected at random for further analysis. Their insert size, determined by gel electrophoresis, was shown to range approximately from 140 to 180 bp, suggesting significant variability between monomers (data not shown). This observation was further supported by the sequence analysis.

Quantitation and Copy Number in the Genome

The relative abundance of the *Alu*I repetitive sequence was assessed from dot-blot experiments. Increasing amounts of a cloned monomer (pMcCo14) and *M. chitwoodi* genomic DNA were blotted onto nitrocellulose membrane and hybridized with the DNA insert released from the same clone (Fig. 2). PUC19 was used as a background control and *Caenorhabditis elegans, Drosophila melanogaster,* and calf thymus DNAs were used as negative controls (data not shown).

The satellite DNA family appears to make up to 3.5% of the nematode genomic DNA, as calculated from values obtained by scintillation measurements. Assuming that the haploid genome size of *Meloidogyne* is about 51 Mb (Pableo and Triantaphyllou 1989), and based on an average monomer size of 156 bp, this fraction corresponds to approximately 11,400 copies per haploid genome.

Primary Structure of the Satellite Monomeric Units

The nucleotide sequence from each of the 19 previously selected monomers was determined (Fig. 3). The main features of these sequences are listed in Table 1. Their lengths were found to be variable, ranging from 142 to 180 bp, thus confirming previous estimations based on the electrophoretic mobility of the inserts. The A + T

Fig. 3. Nucleotide sequences of 19 randomly cloned *Meloidogyne chitwoodi Alu*I satellite monomers, and derived consensus sequences. Ambiguous positions in the consensus are indicated according to the IUB single-letter code. In the monomer sequences, bases showing no variation from their respective consensus are shown with asterisks (*), deletions are indicated with dashes (–), and insertions are shown below the monomer sequences. The restriction sites for *Dra*I (TTTAAA) and *Sau*3A (GATC) are underlined.

Table 1. Comparative features of 19 monomers from the satellite DNA family of *Meloidogyne chitwoodi*

Monomers	Length	%(A + T)	Restriction sites[a] *Dra*I	Restriction sites[a] *Sau*3A	Sub-family
pMcCo2	146	74.7	++	–	1a
pMcCo19	145	74.5	++	–	1a
pMcCo25	145	75.2	++	–	1a
pMcCo112	145	75.2	++	–	1a
pMcCo113	146	76.7	++	–	1a
pMcCo8	143	68.5	+	+	1b
pMcCo14	142	67.6	+	+	1b
pMcCo7	169	66.3	+	–	1c
pMcCo11	169	65.7	+	–	1c
pMcCo28	170	66.5	+	–	1c
pMcCo12	147	69.4	+	–	1d
pMcCo16	144	70.8	+	–	1d
pMcCo120	155	68.4	–	–	2a
pMcCo121	152	69.1	–	–	2a
pMcCo123	152	69.1	–	–	2a
pMcCo101	177	79.1	+	+	2b
pMcCo114	180	78.9	++	+	2b
pMcCo118	169	78.7	++	+	2b
pMcCo122	169	78.1	+	+	2b

[a] –, +, or ++ = no, one, or two restriction sites, respectively

content of the *Alu*I satellite DNA family ranges from 65.7 to 79.1%, with frequent runs of As and Ts. Sequences were also analyzed with respect to the distribution of other putative restriction sites, and computer analyses revealed the occurrence of both *Dra*I and *Sau*3A recognition sites. At least one *Dra*I site was present in most of the sequenced monomers (16/19), whereas *Sau*3A sites were less frequent (6/19). It is to be noted that all clones that had a *Sau*3A site also had at least one *Dra*I site.

The pairwise genetic distances between the 19 cloned sequences were calculated according to Kimura's two-parameter method (1980). The resulting data were used to construct an unrooted neighbor-joining tree of relationships of the pMcCo*n* sequences, according to the method of Saitou and Nei (1987). As could be expected from the comparison of the sequence data, the repeat units appeared to be clustered in two main groups, each one being divided into several heterogenous subgroups (Fig. 4). Sequence length of the monomers, their A + T content, and the presence of *Dra*I and/or *Sau*3A restriction sites proved to be highly conserved between monomers of the same subgroup, as shown in Table 1.

Analysis of Consensus Sequences

Except for four nucleotide positions, an unambiguous consensus sequence was deduced from multiple sequence alignments within each of the six subfamilies previously identified (Fig. 5). The number of variable positions within each subfamily was low but with significant variations among subfamilies, ranging from 1.4 to 12.8% (Table 2). The differences are mainly single nucleotide substitutions and very short insertions or deletions (one to three nucleotides), with the exception of subfamily 2b, for which a deletion of 9 bp (TTGAATTCT) is observed in two repeats (pMcCo101 and pMcCo114). Multiple alignment of the six consensus sequences did not enable the establishment of a general unambiguous consensus sequence (only 22 positions were invariably conserved among the six consensus sequences) (Fig. 6). Although this result is in full agreement with the high level of variation of this repetitive DNA family, blocks of conserved regions could be observed within both family 1 and family 2, with 56 and 92 positions conserved, respectively. Moreover, in the case of family 1, for which four consensus sequences were compared, 57 positions diverged because of a single nucleotide substitution in only one of the four sequences (Fig. 6).

Screening of the six consensus sequences for evidence of internal repeated subunits revealed a 6-bp motif (AAATTT), present in both direct and inverted orientation, which could be found from one to six times in the sequences (Fig. 5). A more careful analysis allowed the detection of additional 6-bp degenerated repeats, differing just by one bp from the AAATTT sequence (Fig. 5). For example, in consensus 1a, the AAATTT motif is present 6 times in perfect repeats plus 3 more times in imperfect repeats. Therefore, the occurrence of this AAATTT repeat is presumably more frequent than on a random basis.

Sequence comparison with EMBL and GenBank nucleic acid databases revealed no significant homology with any recorded sequence for each of the six consensus monomers, which suggested that the pMcCo*n* family represents a novel satellite DNA family.

Discussion

Comparative analysis of satellite DNAs from closely related organisms can be a relevant tool to infer not only the evolution of the satellite itself, but also relationships among species as well (Bachmann et al. 1993). To provide further insight into the genetic diversification of nematodes of the genus *Meloidogyne,* the study of the organization of their genome has been initiated. In this report, we have identified and analyzed the molecular features of an *Alu*I satellite DNA family from the meiotic parthenogenetic species *M. chitwoodi.* The estimation of the reiteration frequency of this DNA class is around 11,400 copies per haploid genome, which corresponds to about 3.5% of the nematode genomic DNA. This represents a low proportion of the genome as compared with other satellite DNAs found in nematodes, for example,

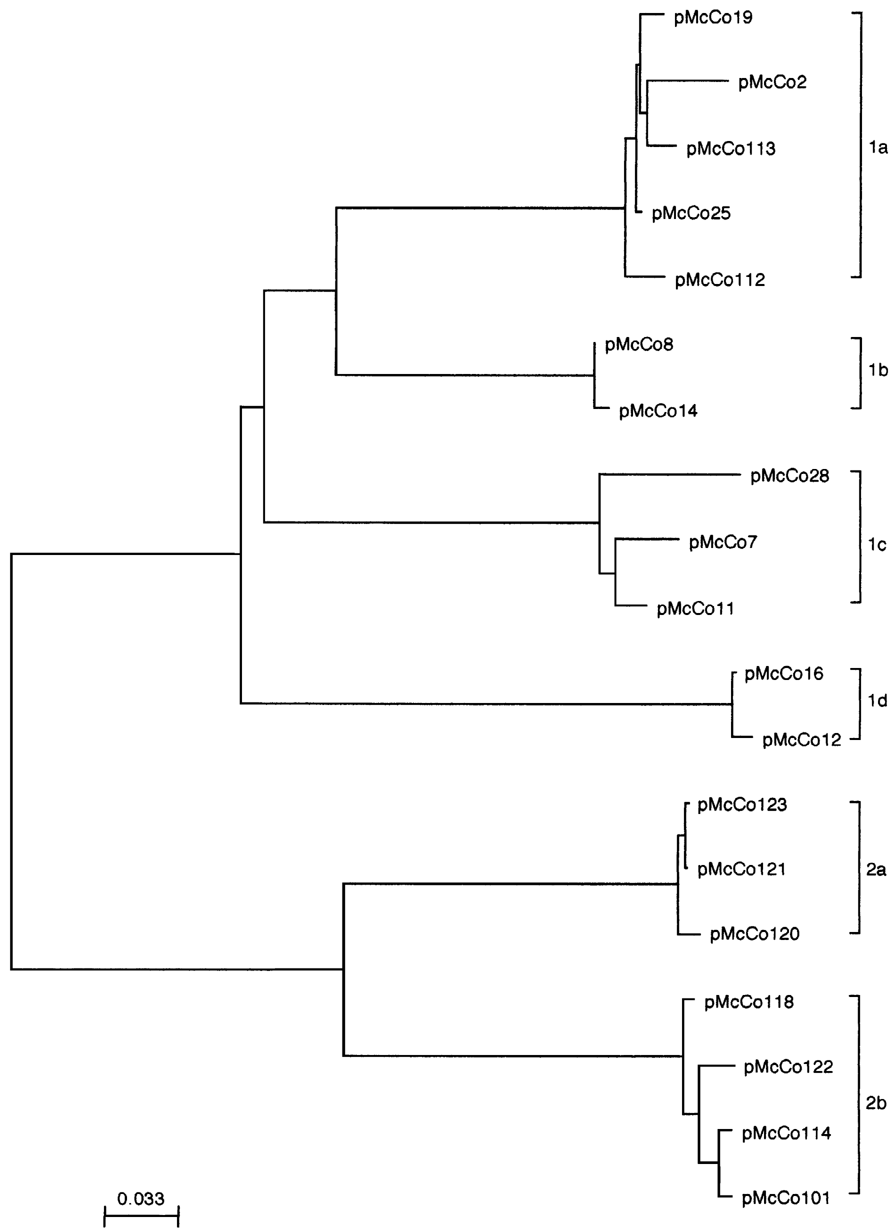

Fig. 4. Unrooted neighbor-joining dendrogram of relationships between the nucleotide sequences of 19 randomly cloned monomers of the *Alu*I satellite DNA family of *Meloidogyne chitwoodi.* Scale bar represents a genetic distance D of 0.033 as the frequency of nucleotide substitutions in pairwise comparison of two sequences according to Kimura's (1980) two-parameter method.

30% in *Bursaphelenchus xylophilus* (Tarès et al. 1993) and 80% in *Parascaris equorum* (Moritz and Roth 1976). In the latter case, it is nevertheless worth considering that the value was obtained from a germ line stock, from which most of satellite DNA is eliminated through the process of chromatin diminution (Müller et al. 1982). However, the percentage estimated for *M. chitwoodi* is in good agreement with what we previously found for the closely related species *M. hapla,* where satellite DNA constitutes 5% of the genome (Piotte et al. 1994).

The 19 monomers of the *Alu*I satellite DNA family that were sequenced in this study have in common a high A + T content (ranging from 65.7 to 79.1%) and share the presence of short stretches of consecutive A and T base pairs. Such features have already been reported in a number of organisms, and seem also to be quite the rule within the Nematoda phylum (Tarès et al. 1993; Callaghan and Beth 1994; Burrows et al. 1995; Grenier et al. 1995). Moreover, the A + T content of the satellite DNA characterized within the closely related species *M. hapla* was evaluated at 68% (Piotte et al. 1994), which is similar to the percentages observed in this study. It has been suggested that during the evolution of satellite DNAs, C-to-T and G-to-A transitions are more frequent than the reverse transitions, which results in A + T enrichment of these sequences (Ugarkovic et al. 1989; Rojas-Rousse et al. 1993). In this hypothesis, the high A + T content observed within this *M. chitwoodi* satellite DNA family strongly suggests it is of ancient origin.

Because of the random sampling of the 19 monomers that were sequenced, we could expect that the range of nucleotide diversity they displayed is representative of the satellite DNA family. Sequence data indicated a high level of variability among the members of this repetitive

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cons 1a   CHTTCGAATG ATACCAAATT CAGCAAATTT CCAACGATGG AAATTTTTTT ATCGAACTTT GAATTATTCC TTCAACCCCA
          AATTTTTAAA ATTTTCAAAA ATTTTTTTCT CAAAAACTAG TCTATAGATT TTTAAATTTC ATAGCT

cons 1b   CTACAAATGA TCCAAATTAC AGCGAATATG ATTCGGAGGA AATTTTTTTA TCGAAGTTTT AAATATTCCT TCCCCCAAAA
          AATTCCAATG YTCATGGACT TGGTTCTCAA AAACTAGTCA TACGATTTTT GATTTTCATA GCT

cons 1c   TGTTCGATTC ACCTCTTCAT CCTCTTTCGA ATGAGATATG ACTCATCCAT AACTTACTTA CAGAATTTTT TTTAAAAAAC
          TCAAAGTATC CATTCTCCCC CCAAAAAATT TCTATGTTCC GACCCCTGTT TCTCAAAAAC TAGTCATACG ATTTTTGAAT
          TTCATAGCT

cons 1d   CTTTCGAATG ATACTAAATT CAGTATACTT TCTCAGAGGG AAAAAAAATT TCTTCAATTT AAAAAATTTT TCTCCCCCCA
          AAAAAATTTC TATGTTCATG AACCTGTTTC TCGAGTTCTG TCTGTAAGTT GCTTGATTTC AATAGCT

cons 2a   GATGAATATT TTTGAAGAAA ATTCAGTAAG TTATGAGACT TGTTCCGAAA ATTTCAGACT TGTTCCGAAA AAATAATTTG
          GGAGGTTCAA CCGCGGCTAA CTTATTATCG ACAAAAATAT TTCTATATGA TTCATATATC ATTCGAAAGA GCT

cons 2b   TTGAATTCTG AATATAAWTA AGTAAGAWTA AACTCTTTTG AACAAAATAC AGATGAGAGT AATGGACTTA TGAAATTGTA
          GGTCAGTTAT TTAAAAAAAA AATTTTGGAA TTTTCAAAAA GATCTAAAAG TTTTTAAAAA AAATTATTAC TTTATGATTC
          ATATATCATT CGAAAGAGCT
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Fig. 5. Consensus sequences of the six subfamilies of the *Alu*I satellite DNA of *Meloidogyne chitwoodi.* Ambiguous positions are indicated according to the IUB single-letter code. The AAATTT internal subrepeats are shown with white boxes. Grey boxes indicate the imperfect repeats. Inverted subrepeats are underlined.

Table 2. Percent variable positions compared with the consensus sequence within each subfamily

Subfamily	Length of the consensus (bp)	% Variable positions
1a	146	8.2
1b	143	1.4
1c	169	9.5
1d	147	2.0
2a	153	2.6
2b	180	12.8

DNA family, and pairwise alignments resulted in nucleotide divergences up to 50% (data not shown). This lack of overall homogeneity in primary structure strongly contrasts with what has been reported for most of the satellite DNAs described so far (Bachmann et al. 1993). In the case of satellite DNAs isolated from nematode genomes, a low level of nucleotide sequence divergence has generally been found: 3% for *M. hapla* (Piotte et al. 1994), 3.9% for *Bursaphelenchus xylophilus* (Tarès et al. 1993), 6% for *Steinernema carpocapsae* (Grenier et al. 1995). Based on sequence comparisons, variant classes were defined for *Ascaris lumbricoides,* but variations were mainly single base substitutions, or in few cases, small deletions or insertions (Müller et al. 1982). On the contrary, the unusual primary structure heterogeneity observed in *M. chitwoodi* satellite DNA is not distributed on a random basis, but is associated with a strong structuring, since the repeated units could be unambiguously clustered in six subfamilies after multiple alignment analysis. Moreover, A + T content, presence of conserved *Dra*I and *Sau*3A restriction sites, and length of the units are in good agreement with the clustering based on primary structure data. From that point of view, the strongly structured variability of this satellite DNA family should be considered as an original result regarding satellite DNA families characterized so far in the Nematoda phylum. In contrast, a high level of sequence homogeneity has been observed within each subfamily. The sequences of all analyzed monomers within a given subfamily appeared to be largely similar, displaying nucleotide substitutions and/or single-nucleotide insertions or deletions as the predominant deviation from the consensus. In most cases, changes seemed to randomly spread among the monomers, thus indicating a series of unrelated mutational events rather than partial homogenization of the sequences.

How can such an unexpected and strongly structured variability within this satellite DNA family be explained? With respect to the model developed by MacGregor and Sessions (1986), satellites accumulate initially around centromeres in large tandem blocks, which later become broken up and dispersed, first to pericentric locations and then gradually more distally into the chromosome arms and telomeres. Due to tandem duplications, a newly appearing satellite family should present a high sequence homogeneity, and its further dispersal should be accompanied by the accumulation of progressive changes in the primary structure of the monomers (e.g., base substitutions and deletions). Nevertheless, some gene conversion mechanisms may act, at the same time, upon the genome to maintain the uniformity of the satellite family (Dover 1982a; Davis and Wyatt 1989). In this connection, our data seem to indicate that the *Alu*I DNA family isolated from *M. chitwoodi* is an ''ancient,'' highly degenerated satellite, presumably located in het-

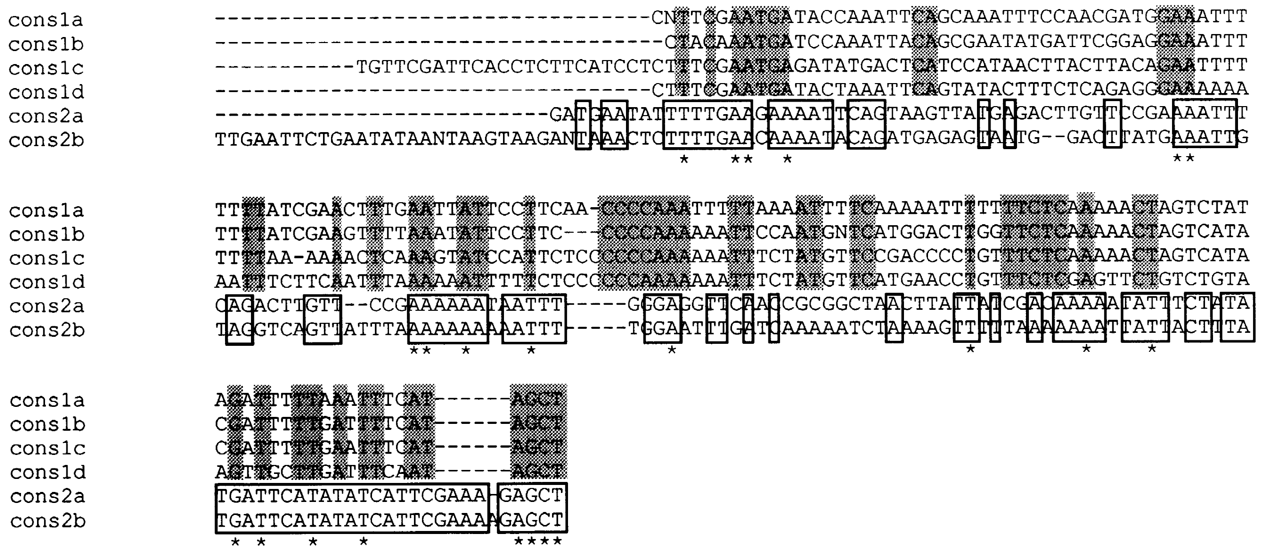

Fig. 6. Alignement of the consensus sequences of the six subfamilies of the *Alu*I satellite DNA of *Meloidogyne chitwoodi.* Asterisks indicate nucleotides identical in all six consensus sequences. Grey and white boxes show the nucleotides conserved in consensus sequences from family 1 and 2, respectively. Ambiguous positions are given according to the IUB single-letter code.

erochromatic regions near the telomeres. In situ localization of the sequences on the nematode chromosomes could be of great interest to confirm this hypothesis, but such an investigation is made impossible by the small size and high condensation of *Meloidogyne* chromosomes, which precludes any clear observation of their shape (Dalmasso pers. comm.). A comparative analysis of the consensus sequences defined for each of the six subfamilies has provided evidence for an internal AAATTT subrepeat, present, with minor variations, from three up to nine times in the consensus. Even if no clear arrangement pattern of these subrepeats could be found, such an occurrence within the 19 basic units argues for the evolution of the *M. chitwoodi Alu*I satellite family from a common ancestral sequence. To simultaneously take into account the sequence heterogeneity between subfamilies and the different levels of sequence homogeneity within each subfamily, it may be proposed that the actual organization of the satellite DNA family could have arisen by successive amplification steps followed by mechanisms of sequence divergence, as it has already been advanced for other classes of highly reiterated sequences (Brutlag 1980; Ugarkovic et al. 1996). In this respect, each subfamily could result from a different amplification event, and sequence variations within them could either reflect the existence of distinct rates of gene conversion, or could represent different transition stages in the process of sequence homogenization (Plohl et al. 1992). Although no data concerning the datation of these putative amplification steps are presently available, some indirect arguments may indicate differences in the relative age of the six subfamilies. For example, among the six subgroups defined, A + T content and % of positions not conserved relative to the consensus sequence are significantly higher within subfamily 2b, thus suggesting that it arose and spread earlier than the other subfamilies.

An alternative view to this evolutionary pathway could be proposed considering that the mode of reproduction (i.e., parthenogenesis vs amphimixis) can change repeatedly during the evolution of a species, which is highly likely in the case of the genus *Meloidogyne.* Based on cytogenetic information, there is evidence that ancestral root-knot nematodes were amphimictic animals, and that parthenogenetic forms (i.e., the most frequently encountered today) evolved from amphimictic ones (Triantaphyllou 1985). In that respect, the ancestral sequence of the *Alu*I satellite DNA family could have evolved independently in isolated amphimictic *M. chitwoodi* populations, and the six subfamily pattern observed within the isolate studied here could be the result of subsequent hybridization events between these populations. Such gene flow between ancestral amphimictic nematodes could explain the current occurrence of different (and more or less related) subfamilies within the genome of a single *M. chitwoodi* population.

Even if no clear function has been demonstrated for satellite DNAs, it has been proposed that they may play a structural role in chromosome spatial organization and location during mitosis and meiosis (Brutlag 1980). In this sense, they could prevent any abnormal chromosome pairing, and therefore be involved in maintaining the reproductive isolation of species. This could be illustrated by the fact that most of the satellite DNAs cloned so far from nematode genomes have been described to be species specific and cannot be detected even in sibling species (Abad 1994). In preliminary experiments, no cross-hybridization was observed with the closely related species *M. hapla, M. incognita,* and *M. javanica* when using *Alu*I satellite monomers as a probe, thus suggesting the species specificity of these sequences (data not

shown). Therefore, and whatever its evolutionary pathway could be, the *Alu*I satellite DNA family we have characterized could represent some trace of the time when the *Meloidogyne* were amphimictic organisms. Consequently, and in good agreement with previous cytogenetic (Triantaphyllou 1985) and molecular data (Adams and Powers 1996), all our observations are consistent with the suggestion that the species *M. chitwoodi* should hold a position close to the ancestral forms of root-knot nematodes.

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