GH gene in various animals. A glucocorticoid regulatory element (GRE), which may be responsible for the transcriptional control of the human GH (hGH) gene, has been located in the first intron of the hGH gene (Moore et al. 1985; Slater et al. 1985). A pituitary-specific transcription factor, GHF1, which was suggested to be involved in the tissue-specific expression of the GH gene has also been identified in intron 3 of the rainbow trout GH gene (Bernardini et al. 1999). These investigations suggest that introns in the GH gene might play a crucial role in the regulation of GH gene expression.

Previous studies comparing the genomic cGH sequence in White Leghorn to the Chinese Yellow Wai Chow strain demonstrated a total of 32 substitutions and additions (Ip et al. 2001). Although most substitutions were identified in introns 1 and 4, a silent substitution was observed in exon 2. Compared to results of intron 1 polymorphism, the results on intron 4 polymorphism also demonstrated that Chinese native chickens display a much wider variety of alleles. Previous studies on allele frequencies of intron 1 polymorphism at *Msp*I sites showed that there were significant differences between Chinese chickens and layer chickens (Ip et al. 2001). Although no difference has been observed in the allele frequencies at *Msp*I sites among Chinese native chickens, broilers, and layers, two more alleles were identified in Chinese native chickens and its hybrids, but not in broilers and layers. Of these alleles, allele *D* was found in Taihe Silkies and Beijing Fatty, and other native breeds only. Taihe Silkies, a silkies breed, has silkies feathers and black skin, while Beijing Fatty has a feathered shank and comb (Qiu et al. 1988). Of interest is that both are slow growing. However, whether allele *D* is associated with slow growth rate or silkies feathers requires further study.

Allele *E,* which was characterized by a deletion of 50 bp from position 421 to 470, was identified in the Taihe Silkies population only. Deletion of the 50 bp fragment not only reduces the size of the PCR fragment (Figure 1b), but also, in addition to the introduction of an *Msp*I site at 510 nt, forms a new fragment of 460 bp. Previous studies based on Northern and Southern blotting and PCR analysis revealed RFLP patterns of a 1.7 kb deletion of the intracellular domain of the GH receptor gene in dwarf broiler chickens (Agarwal et al. 1994). Deletion in the GH receptor gene also leads to decreasing muscle cell proliferation in chickens (Goddard et al. 1996). Recent studies on the genomic structure of the gilthead seabream GH gene also identified a length polymorphism in the first intron, which is due to differences in the number of repeat monomers (Almuly et al. 2000). As this 50 bp deletion was observed only in the Taihe Silkies population, whether the 50 bp deletion allele was associated with other phenotypes remains to be clarified in the future.

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References

Agarwal SK, Cogburn LA, Burnside J, 1994. Dysfunctional growth hormone receptor in a strain of sex-linked dwarf chicken: evidence for a mutation in the intracellular domain. J Endocrinol 142:427–434.

Almuly R, Cavari B, Ferstman H, Kolodny O, and Funkenstein B, 2000. Genomic structure and sequence of the gilthead seabream (*Sparus aurata*) growth hormone-encoding gene: identification of minisatellite polymorphism in intron I. Genome 43:836–845.

Bernardini S, Argenton F, Vianello S, Colombo L, and Bortolussi M, 1999. Regulatory regions in the promoter and third intron of the growth hormone gene in rainbow trout, *Oncorhynchus mykiss* Walbaum. Gen Comp Endocrinol 116:261–271.

Byatt JC, Staten NR, Salsgiver WJ, Kostele JC, and Collier RJ, 1993. Stimulation of food intake and weight gain in mature female rats by bovine prolactin and bovine growth hormone. Am J Physiol 264:986–992.

Copras E, Harman SM, and Blackman MR, 1993. Human growth hormone and human aging. Endocr Rev 14:20–39.

Goddard C, Johnson R, Gilhooley HJ, Gardner JO, Gray A, Wilkie RS, and Butterwith SC, 1996. Decreased muscle cell proliferation in chicks with a deletion in the GH receptor gene. J Mol Endocrinol 17:61–78.

Ip SC, Zhang X, and Leung FC, 2001. Genomic growth hormone gene polymorphism in native Chinese chicken. Exp Biol Med 226:458–462.

Kuhnlein U, Liu N, Weigend S, Gavora JS, Fairfull W, and Zadworny D, 1997. DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Anim Genet 28:116–123.

Lagziel A, Lipkin E, Ezra E, Soller M, and Weller JI, 1999. An *Msp*I polymorphism at the bovine growth hormone (bGH) gene is linked to a locus affecting milk protein percentage. Anim Genet 30:296–299.

Lechniak D, Machnik G, Szydlowski M, and Switonski M, 1999. Growth hormone gene polymorphism and reproductive performance in AI bulls. Theriogenology 52: 1145–1152.

Moore DD, Marks AR, Buckley DI, Kapler G, Payvar F, and Goodman HM, 1985. The first intron of the human growth hormone gene contains a binding site for glucocorticoid receptor. Proc Natl Acad Sci USA 82:699–702.

Mou L, Liu N, Zadworny D, Chalifour L, and Kuhnlein U, 1995. Presence of an additional *Pst*I fragment in intron 1 of the intron 1 of the chicken growth hormone-encoding gene. Gene 160:313–314.

Qiu X, 1988. Poultry breeds in China. Shanghai: Shanghai Science and Technology Press; 1–82.

Slater EP, Rabenau O, Karin M, Baxter JD, and Beato M, 1985. Glucocorticoid receptor binding and activation of a heterologous promoter by dexamethasone by the first intron of the human growth hormone gene. Mol Cell Biol 5:2984–2992.

Tanaka M, Hosokawa Y, Watahiki M, and Nakashima K, 1992. Structure of the chicken growth hormone-encoding gene and its promoter region. Gene 112:235–239.

Vasilatos-Younken R, Dunnington EA, Siegel PB, and McMurtry JP, 1997. Tissue-specific alterations in insulin-like growth factor-1 concentrations in response to 3,3′,5-triiod-L-thyronine supplementation in the growth hormone receptor-deficient sex-linked dwarf chicken. Gen Comp Endocrinol 105:31–39.

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rDNA (18S–28S and 5S) Colocalization and Linkage Between Ribosomal Genes and $(TTAGGG)_n$ Telomeric Sequence in the Earthworm, *Octodrilus complanatus* (Annelida: Oligochaeta: Lumbricidae), Revealed by Single- and Double-Color FISH

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Spermatogonial and metaphase I chromosomes of the lumbricid earthworm *Octodrilus complanatus* (Annelida: Oligochaeta) were examined using fluorescent in situ hybridization (FISH) with three repetitive DNA probes—5S rDNA, 18S–26S rDNA, and $(TTAGGG)_n$. Single-color FISH consistently mapped one chromosome pair per spread using either 5S rDNA or 18S–26S rDNA as probes. Simultaneous (18S–26S)-5S and (18S–26S)-$(TTAGGG)_n$ FISH demonstrated that repeated units of the two ribosomal families were overlapped and closely associated with telomeric sequences.

The genes coding for rRNA in eukaryotes are arranged into two different rDNA multicopy families, that is, major (18S, 5.8S, and 28S rDNA) and minor (5S rDNA) ribosomal genes. The two rDNAs, detectable as chromosomal hybridized labels, were found to be located at different chro-

mosome pairs as the most frequent configuration in vertebrates (Lucchini et al. 1993; Suzuki et al. 1996). However, 5S units have also been found to be associated with repeated copies of major rDNA (Drouin and Moniz de Sá 1995; Mandrioli et al. 2000; Pendás et al. 1994) or histone genes (Andrews et al. 1987; Barzotti et al. 2000).

Fluorescent in situ hybridization (FISH) with ribosomal sequences as probes allowed the mapping of 18S–28S and 5S rDNA genes on the chromosomes of several vertebrate species. In contrast, invertebrate taxa remained poorly characterized in this respect. For example, in the class Oligochaeta, major rDNA genes have been mapped using single-color FISH to spermatocyte chromosomes of two lumbricid species, *Eisenia foetida* and *Octodrilus complanatus* (Vitturi et al. 2000a), whereas to the authors' knowledge, nothing is known about the 5S rDNA localization among these organisms.

In the present work we mapped for the first time in Oligochaeta the 5S rDNA to the chromosomes of *Octodrilus complanatus* using single-color FISH. Moreover, since next to nothing or very little is known about the mapping of repetitive DNAs in the oligochete earthworms, we used, in addition to single-color FISH, double-color FISH to examine the physical relationship between 18S–28S rDNA, 5S rDNA, and $(TTAGGG)_n$ telomeric repeats.

O. complanatus is a lumbricid earthworm of the class Oligochaeta. Its chromosome complement ($2n = 36$) consists of seven metacentric/submetacentric pairs (M/SM) and 11 subtelocentric pairs (ST) homogeneously fluorescent after CMA_3 and DAPI staining. FISH using a sea urchin (*Paracentrotus lividus*) 18S–26S ribosomal probe mapped major rDNA genes on one small subtelocentric chromosome pair (Vitturi et al. 2000a). In the same study the $(TTAGGG)_n$ FISH showed that the hexanucleotide hybridized to the ends of the chromosome of *E. foetida* and *O. complanatus.*

Materials and Methods

A total of 20 *O. complanatus* (Dugés 1828) specimens were collected at the Botanic Garden of Palermo (Sicily). Chromosomes were prepared by means of the air-drying technique (Vitturi et al. 1991) from dissected testes of sexually mature specimens previously soaked in a freshwater colchicine solution (0.1%) at room temperature.

FISH was performed on fixed spermatocyte chromosomes of five specimens as described by Vitturi et al. (2000a) using three different probes: a 4.3 kb sea urchin (*Paracentrotus lividus*) rDNA probe (prR14) consisting of sequences from the 3′ end of the 18S to the 3′ end of the 26S rDNA (Cantone et al. 1993); a PCR-obtained 5S rDNA probe using as primers F (5′-TGCACGTAGTGTTCCCAAGC) and R (5′-ACGACCATACCACGTTGAATAC), deduced from the 5S coding sequence of insects available in GenBank, according to the protocol described by Mandrioli et al. (2000); and a telomeric hexanucleotide $(TTAGGG)_n$ generated by PCR (PCR DIG-probe Synthesis Kit, Roche) in the absence of template (Ijdo et al. 1991) using $(TTAGGG)_5$ and $(CCCTAA)_5$ as primers. For single-color FISH, nick translation labeling with digoxigenin of 18S–26S rDNA was performed according to the manufacturer's (Boehringer Mannheim) instructions, while the remaining two probes were DIG-labeled following the Roche protocol.

Slides treated with single-color FISH were mounted in an antifade solution containing propidium iodide (5 μm/ml) and viewed under a Leica I3 filter set (BP 450–490, LP 515). For multiple FISH the 18S–26S rDNA probe was labeled with biotin (Biotin-Nick Translation Mix, Roche), whereas both 5S rDNA and $(TTAGGG)_n$ probes were the same as those used in single-color experiments.

Chromosomes were denatured for 4 min in 70% formamide/2× SSC at 72°C. The mixed probe solution (4 ng/μl of each probe) was denatured for 5 min at 80°C. Hybridization was allowed to proceed in a moist plastic chamber at 37°C overnight. Slides were washed twice in 50% formamide/2× SSC at 37–38°C (5 min each), twice in 2× SSC at 37–38°C (5 min each), once (5 min) in 4× SSC/0.1% Tween at RT, and finally, once (5 min) in PBS/0.1% Tween/0.5% skim milk powder at RT. Probe hybridization sites were detected using both anti-DIG-fluorescein, Fab fragments, and streptavidin-Texas Red conjugate, according to the manufacturer's (Boehringer Mannheim, molecular probes) instructions. Slides were mounted in an antifade solution containing DAPI (3 μg/ml) and viewed under a Leica three-color filter set (B/G/R filter set, BP 400/20, BP 465/20) which allowed the simultaneous visualization of fluorescein- and Texas Red-labeled hybridization sites (green and red, respectively) and chromosomal DNA (blue). Chromosomes were observed with a Leica microscope and photographed on with Kodak Ektacolor 1000 ASA film.

Results

As was reported in a previous article (Vitturi et al. 2000a), in this study as well, single-color 18S–26S rDNA FISH showed that in *O. complanatus,* two hybridization sites per spread occurred at spermatogonial metaphase (Figure 1a). Two positive sites per spread were also obtained using single-color FISH with the 5S rDNA as a probe (Figure 1b). Both major (18S–28S rDNA) (Figure 1a) and minor (5S rDNA) (Figure 1b) ribosomal genes were located at the terminal region of two small sized chromosomes.

After single-color FISH with either the 18S–26S (Figure 1c) or 5S (Figure 1d) rDNA as a probe, a single metaphase I bivalent showed ribosomal sites, thus demonstrating that repeated units of major and minor rDNA were located at homologous chromosomes. Simultaneous double-color FISH was performed on diakinetic chromosomes of *O. complanatus* using the biotin-labeled 18S–26S rDNA and the digoxigenin-labeled $(TTAGGG)_n$ as probes. Telomeric signals, probably due to their small dimension, were not clearly distinguishable in all bivalents. In spite of this it was possible to establish that ribosomal (red spot) and telomeric (green spot) sequences were located adjacently in the rDNA-bearing bivalent (Figure 1e).

After simultaneous double-color FISH using the biotin-labeled 18S–26S rDNA and the digoxigenin-labeled 5S rDNA as probes, two hybridized chromosomes were obtained at spermatogonial metaphase (Figure 1f) and one hybridized bivalent at metaphase I (Figure 1g). Hybridization sites showed a mixed-color appearance. Analysis of these sites using a spot software diagnostic instrument for decomposition of colors revealed that each hybridization spot (Figure 1g) was the result of the overlapping of two distinct colors, namely red (Figure 1h) and green (Figure 1i).

Discussion

Cytological studies in the class Oligochaeta are predominantly restricted to chromosome counts of about 160 species and to gross karyotype description of some of them (Christensen 1980 and references therein). Conventional banding techniques, such as silver staining (Ag-NOR) and C-banding, have been attempted on spermatogonial chromosomes of the lumbricid *Eisenia foetida* (Vitturi et al. 1991). Recently single-color FISH has been used

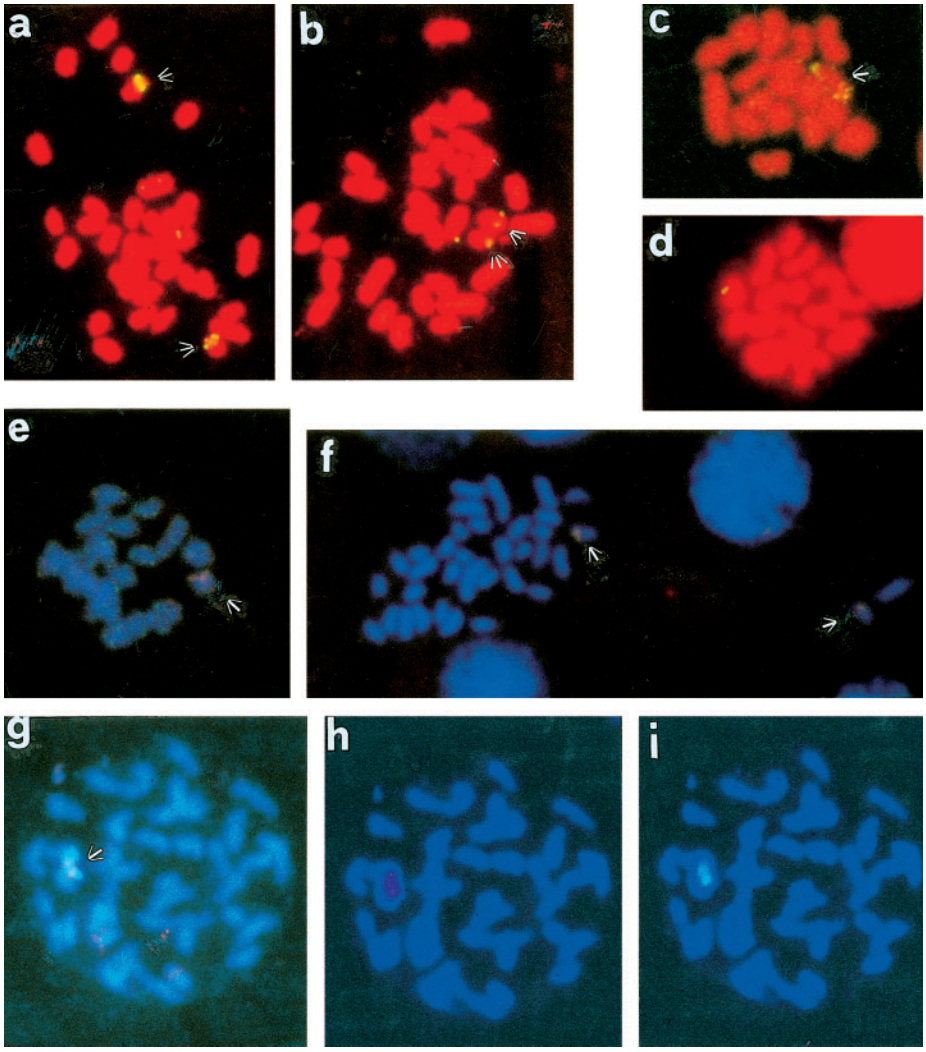

Figure 1. Chromosome spreads of *Octodrilus complanatus.* Single-color 18S–26S rDNA FISH to spermatogonial chromosomes (arrows indicate major ribosomal sites). **(a)** Single-color 5S rDNA FISH to spermatogonial chromosomes (arrows indicate minor ribosomal sites). **(b)** Single-color 18S–26S **(c)** and 5S **(d)** rDNA FISH to metaphase I bivalents (arrow indicates the hybridized bivalent). Double-color FISH using the biotin-labeled 18S–26S rDNA and the digoxigenin-labeled $(TTAGGG)_n$ telomeric sequence (arrow indicates the hybridized bivalent) **(e).** Double-color FISH using the biotin-labeled 18S–26S rDNA (arrows indicate the two hybridized chromosomes at spermatogonial metaphase) **(f)** and the hybridized bivalent at metaphase I **(g).** Hybridization signal **(h)** decomposed in a red spot (18S–28S rDNA) and a green spot (5S rDNA) **(i)** using a spot software diagnostic instrument.

to map major rDNA genes (18S–28S) and to test the presence of the $(TTAGGG)_n$ telomeric repeats in the lumbricid species *E. foetida* and *O. complanatus* (Vitturi et al. 2000a).

In the present article, the single-color FISH has been used for the first time to map the 5S rDNA on the chromosomes of *O. complanatus.* In addition, simultaneous double-color FISH in combination with DAPI counterstaining has been used to investigate the relationship between repeated units of three multigene families, namely 18S–28S rDNA, 5S rDNA, and $(TTAGGG)_n$ telomeric sequences.

The results obtained with single-color FISH demonstrate that in *O. complanatus,* the 5S and 18S–26S rDNA hybridized to the short arms of one small-size chromosome pair. However, this technique did not allow establishment of whether the hybridized pair was the same in the two experiments. Double-color FISH conclusively demonstrated that in *O. complanatus,* the 5S and 18S–28S sites were overlapped. This assumption was deduced from two main arguments. One argument is that the use of two different probes in the same experiment gave a single hybridization signal. A second argument is that the analysis of hybridization sites using a spot software for decomposition of colors showed that each signal was the result of the overlapping of two spots, red (18S–28S rDNA) and green (5S rDNA).

Potentially functional 5S rRNA-encoding genes were found interspersed with the rDNA repeats in the nematode *Meloidogyne arenaria* (Vahidi et al. 1991) and with histone genes in the crustacean *Artemia* spp. (Andrews et al. 1987) and *Asellus aquaticus* (Barzotti et al. 2000). Moreover, the existence of a colocalization of 5S rDNA and 18S–28S rDNA has been reported in 7 of 11 calanoid copepod species analyzed thus far (Drouin and Moniz de Sá 1995; Drouin et al. 1987, 1992), in the cenogastropod *Melarhaphe neritoides* (Mollusca: Prosobranchia) (Colomba et al. 2002), and in four fish species (Mandrioli et al. 2000; Morán et al. 1996; Pendás et al. 1994).

The double-color FISH was also used to investigate the linkage of the $(TTAGGG)_n$ telomeric sequence and 18S–28S ribosomal repeats. The results revealed that in the NOR-bearing chromosomes of *O. complanatus,* the telomeric signal was closely associated with major ribosomal sequences. Therefore, as recently found in *M. neritoides* (Colomba et al. 2002), and in the earthworm as well, three multicopy families are linked on the same chromosome pair.

By combining the FISH and silver-staining techniques, linkage or interspersion between the $(TTAGGG)_n$ sequences and NORs was found in some vertebrate species including the Eastern American mole (*Scalopus aquaticus*), the wood lemming (*Myopus schisticolor*), two Atlantic eels, and four salmonid species (Abuin et al. 1996; Liu and Fredga 1999; Meyne et al. 1990; Salvadori et al. 1995). The hexanucleotide $(TTAGGG)_n$ was also found at NORs in nonvertebrate species such as *Neurospora, Giardia,* and *Tetrachymena* (Adam et al. 1991; Butler 1992; Yu and Blackburn 1991).

The significance of such an association and/or interspersion involving repeated units of different multigene families is still unclear. However, available literature provides two possible interpretations. One interpretation is that this close association might have a functional role in nucleolus organization, as demonstrated for a human nonribosomal tandemly repeated block of a pentamer sequence located near the NORs (Kaplan et al. 1993).

A second possibility is that the colocalization or interspersion of repeated units of different multigene families might result in unequal crossing-over with consequent heteromorphism which, according to some authors (Dover 1986; Liu and Fredga 1999), is important in the maintenance of a conserved and multiple array. Taking

into account that in most vertebrate species a colocalization of 18S–28S and 5S rDNAs is not the rule, but rather the exception (Mandrioli et al. 2000; Sola et al. 2000), this possibility may be true only for some species.

As far as invertebrates are concerned, where a high degree of NOR polymorphism has been described (Sella et al. 1995 and references therein; Vitturi et al. 2000b), the FISH technique to investigate the relationship between repeated units of multigene families has rarely been used. However, in disagreement with data reported for vertebrates, where an adjacent disposition between NORs and telomeres is unusual (Liu and Fredga 1999), in invertebrate species results obtained using silver staining seem to indicate that major rDNA clusters are closely associated with telomeric sequences. In fact, in most studied species, Ag-NORs were found to be terminal or subterminal.

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References

Abuin M, Martínez P, and Sánchez L, 1996. Localisation of repetitive telomeric sequence $(TTAGGG)_n$ in four salmonid species. Genome 39:1035–1038.

Adam RD, Nash TE, and Wellems TE, 1991. Telomeric location of *Giardia* rDNA genes. Mol Cell Biol 11:3326–3330.

Andrews MT, Vaughn JC, Perry BA, and Bagshaw JC, 1987. Interspersion of histone and 5S genes in *Artemia.* Gene 51:61–67.

Barzotti R, Pelliccia F, Bucciarelli E, and Rocchi A, 2000. Organization, nucleotide sequence, and chromosomal mapping of a tandemly repeated unit containing the four core histone genes and a 5S rRNA gene in an isopod crustacean species. Genome 43:341–345.

Butler DK, 1992. Ribosomal DNA is a site of chromosome breakage in aneuploid strains of *Neurospora.* Genetics 131:581–592.

Cantone M, Barbieri R, Duro G, Izzo V, and Giudice G, 1993. Sequence analysis of the rDNA spacer of *Paracentrotus lividus* and observations about pre-DNA processing. Mol Biol 18:177–182.

Christensen B, 1980. Animal cytogenetics, vol. 2. Stuttgart: B. John.

Colomba MS, Vitturi R, Castriota L, Bertoni R, and Libertini A, 2002. FISH mapping of 18S–28S and 5S ribosomal DNA, $(GATA)_n$ and $(TTAGGG)_n$ telomeric repeats in the periwinkle *Melarhaphe neritoides* (Prosobranchia: Gastropoda: Cenogastropoda). Heredity 88:381–384.

Dover GA, 1986. Linkage disequilibrium and molecular drive in the rDNA gene family. Genetics 122:249–252.

Drouin G, Hofman JD, and Doolittle WF, 1987. Unusual ribosomal RNA gene organization in copepods of the genus *Calanus.* J Mol Biol 196:943–946.

Drouin G and Moniz de Sá M, 1995. The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. Mol Biol Evol 12:481–493.

Drouin G, Sévigny JM, McLaren IA, Hofman JD, and Doolittle WF, 1992. Variable arrangement of 5S ribosomal genes within the ribosomal DNA repeats of arthropods. Mol Biol Evol 9:826–835.

Ijdo JW, Wells RA, Baldini A, and Reeders ST, 1991. Improved telomere detection using a telomere repeat probe $(TTAGGG)_n$ generated by PCR. Nucleic Acids Res 19:4780.

Kaplan FS, Murray J, Sylvester JE, Gonzalez IL, O'Connor P, Doering JL, Muenke M, Emanuel BS, and Zasloff MA, 1993. The topographic organization of repetitive DNA in the human nucleolus. Genomics 15: 123–132.

Liu WS and Fredga K, 1999. Telomeric $(TTAGGG)_n$ sequences are associated with nucleolus organizer regions (NORs) in the wood lemming. Chromosome Res 7:235–240.

Lucchini S, Nardi I, Barsacchi G, Batistoni R, and Andronico F, 1993. Molecular cytogenetics of the ribosomal (18S+28S and 5S) DNA loci in primitive and advanced urodele amphibians. Genome 36:762–773.

Mandrioli M, Colomba MS, and Vitturi R, 2000. Chromosomal analysis of repeated DNAs in the rainbow wrasse *Coris julis* (Pisces, Labridae). Genetica 108:191–195.

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, Wuster-Hill DH, Yates TL, and Moyzis RK, 1990. Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequences in vertebrate chromosomes. Chromosoma 99:3–10.

Morán P, Martínez JL, García-Vázquez E, and Pendás AM, 1996. Sex chromosome linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). Cytogenet Cell Genet 75:145–150.

Pendás AM, Morán P, Freije JP, and García-Vazquez E, 1994. Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA. Cytogenet Cell Genet 67:31–36.

Salvadori S, Deiana AM, Coluccia E, Floridia G, Rossi E, and Zuffardi O, 1995. Colocalization of $(TTAGGG)_n$ telomeric sequences and ribosomal genes in Atlantic eels. Chromosome Res 3:54–58.

Sella G, Vitturi R, Ramella L, and Colomba MS, 1995. Chromosomal nucleolar organizer region (NOR) phenotypes in nine species of the genus *Ophryotrocha* (Polichaeta:Dorvilleidae). Mar Biol 124:425–433.

Sola L, De Innocentiis S, Gornung E, Papalia S, Rossi AR, Marino G, De Marco P, and Cataudella S, 2000. Cytogenetic analaysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18S and 5S rRNA genes and of the $(TTAGGG)_n$ telomeric sequence. Mar Biol 137:47–51.

Suzuki H, Sakurai S, and Matsuda Y, 1996. Rat rDNA spacer sequences and chromosomal assignment of the genes to the extreme terminal region of chromosome 19. Cytogenet Cell Genet 72:1–4.

Vahidi H, Purac A, LeBlanc JM, and Honda BM, 1991. Characterization of potentially functional 5S rRNA-encoding genes within ribosomal DNA repeats of the nematode *Meloidogyne arenaria.* Gene 108:281–284.

Vitturi R, Colomba MS, Pirrone A, and Libertini A, 2000a. Physical mapping of rDNA genes, $(TTAGGG)_n$ telomeric sequence and other karyological features in two earthworms of the family Lumbricidae (Annelida: Oligochaeta). Heredity 85:203–207.

Vitturi R, Colombera D, Catalano E, and Amico FP, 1991. Karyotype analysis, nucleolus organizer regions and C-banding pattern of *Eisenia foetida* (Oligochaeta, Lumbricidae). Genetica 83:159–165.

Vitturi R, Ramella L, Colomba MS, Caputo V, and Sella G, 2000b. NOR regions of polychaete worms of the genus *Ophryotrocha* studied by chromosome banding techniques and FISH. J Hered 91:18–23.

Yu GL and Blackburn EH, 1991. Developmentally programmed healing of the chromosomes by telomerase in *Tetrahymena.* Cell 67:823–832.

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Conservation of $(TTAGG)_n$ Telomeric Sequences Among Ants (Hymenoptera, Formicidae)

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To determine the telomere sequence in *Tapinoma nigerrimum,* we carried out in situ hybridization using TTAGGG and TTAGG repeat polymerase chain reaction (PCR)-generated probes. No hybridization signals were found when TTAGGG was used as a probe. However, strong signals were observed at the end of the chromosomes with the TTAGG probe. Southern blot analysis carried out on genomic DNA using TTAGG as a probe showed a strong hybridization signal even under highly stringent conditions. Similar results were obtained in Southern blot analysis carried out on genomic DNA of 19 species of ants belonging to three different subfamilies. In accordance with all the results shown in this article, the TTAGG repeat seems to be the major component of the telomere sequence in the majority of ant species.

Telomeres are at the end of eukaryotic chromosomes and consist of many tandemly repeated copies of basic short sequences, which are synthesized by the reverse transcriptase activity of telomerase (Blackburn 1991). Telomere sequences are very similar among species, consisting of tandem arrays of simple sequence 3′ G-rich, according to the $(T)_nA(G)_n$ pattern (reviewed in Henderson 1995; Zakian 1995).

In the insect species for which telomere organization has been studied, three different types of organization have been described: a pentanucleotide sequence repeat $(TTAGG)_n$ from the silkworm (*Bombyx mori*) (Okazaki et al. 1993), HeT-A and TART transposable elements in *Drosophila melanogaster* (Diptera) (reviewed in Mason and Biessmann 1995), and long complex and regular tandem repeats in