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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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

chironomids (Diptera) (Rosén and Edström 2000; Zhang et al. 1994). The $(TTAGG)_n$ repeat appears to be a widespread, though not the only telomere DNA motif in insects and other arthropods according to Southern hybridization and fluorescence in situ hybridization (FISH) data (Okazaki et al. 1993; Sahara et al. 1999).

Recently telomerase activity of insects has been detected using a modified telomeric repeat amplification protocol (TRAP) (Sasaki and Fujiwara 2000). This telomerase required dATP, dGTP, and dTTP, but not dCTP, as a substrate and sequence analyses of the products of TRAP revealed that the $(TTAGG)_n$ repeats are synthesized by telomerase. However, in some species which have $(TTAGG)_n$, such as *B. mori,* the absence or very weak activity of telomerase has been reported (Sasaki and Fujiwara 2000).

The telomeric organization of the order Hymenoptera has been studied in only a few species. $(TTAGG)_n$-containing telomeres were found in the honeybee (*Apis mellifera*) (Hymenoptera, Apidae) and in the ant *Manica yessensis* (Hymenoptera, Formicidae) (Okazaki et al. 1993; Sahara et al. 1999). However, telomeric hybridization with the TTAGG oligomers and with the putative vertebrate telomere sequence, TTAGGG, has also been reported in several species of ants belonging to the genus *Myrmecia* (Meyne et al. 1995).

In this article we analyze the telomeric sequences of the ant *Tapinoma nigerrimum* by Southern and in situ hybridization using synthetic oligonucleotides with the sequences $(TTAGGG)_n$ and $(TTAGG)_n$. In addition, we used Southern blotting to determine the presence of the TTAGG and TTAGGG repeats in 19 species of ants included in three subfamilies: Dolichoderinae, Formicinae, and Myrmicinae.

Materials and Methods

Ant Material and DNA Isolation

The ant species examined are indicated in the following figures. Ants were collected in Spain. Adult workers were used for DNA extraction according the technique described by Heinze et al. (1994). *Drosophila melanogaster* and spleens from mice were also used for DNA isolation using the same technique.

DNA Probes Labeling for Southern Analysis

Synthetic oligonucleotides with the sequence $(TTAGGG)_5$ or $(TTAGG)_6$ were directly DIG-labeled using terminal transferase (Roche) according to the instructions of the supplier. Labeled probes were dissolved in hybridization solution (5× SSC, 0.1% N-laurosylsarcosine, 0.02% SDS, 1% blocking reagent) to a final concentration of 2 pmol/ml.

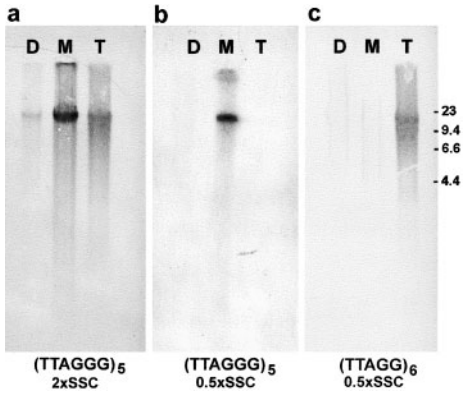

Figure 1. Southern hybridization of *D. melanogaster* (D), mouse (M), and *T. nigerrimum* (T) DNA digested with *Eco*RI using the oligonucleotide **(a,b)** $(TTAGGG)_5$ and **(c)** $(TTAGG)_6$. Hybridizations were performed at 55°C. Posthybridization washes (20 min at 55°C) used the following salt conditions: **(a)** 4× SSC, 2× SSC, **(b,c)** 4× SSC, 2× SSC, 1× SSC, 0.5× SSC. Molecular weight ladder is in kb.

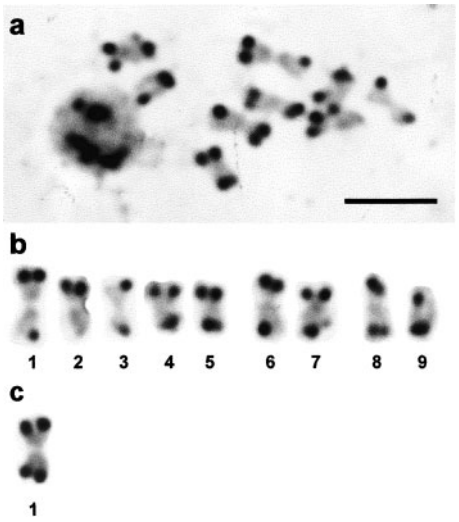

Figure 2. In situ hybridization of *T. nigerrimum* chromosomes using TTAGG repeats PCR-generated probes. **(a)** Metaphase plate and **(b)** karyotype showing strong hybridization signals at the end of the chromosomes. Two chromosomes, **(b)** showing a negative and a **(c)** positive hybridization signal at the same telomere. Bar = 5 μm.

Southern Hybridization

DNA samples (2 μg) digested with *Eco*RI were separated by electrophoresis on a 0.8% agarose gel and blotted to a nitrocellulose filter. Hybridization with oligonucleotides $(TTAGGG)_5$ and $(TTAGG)_6$ was performed overnight at 55°C. Posthybridization washes were carried out in different steps with four different stringency conditions: (1) 2× SSC, (2) 2× + 1× SSC, (3) 2× + 1× + 0.5× SSC, and (4) 2× + 1× + 0.5× + 0.1× SSC, according to the procedure of Okazaki et al. (1993). Each wash was conducted at 55°C for 20 min. Hybridization signals were detected using the alkaline phosphatase DIG-detection kit (Roche).

DNA Probes Labeling for In Situ Hybridization

Telomeric probes were generated by polymerase chain reaction (PCR) using $(TTAGG)_6$ and $(TAACC)_6$ as primers, which when mixed, annealed to two stable double-stranded DNA forms with two base 3′ and three base 5′ protruding ends, respectively. PCR was performed in 100 μl using 100 pmol of each primer and 2.5 U of *Taq* polymerase in the absence of a template. Amplification consisted in 30 cycles, each 1 min at 95°C, 1 min at 50°C, 1 min at 72°C, and a final step of 10 min at 72°C. PCR-generated fragments between 200 bp and 1 kb were labeled with biotin using the biotin labeling kit from Roche. A similar procedure was used to obtain a mammal telomeric probe, using the oligonucleotides $(TTAGGG)_5$ and $(TAACCC)_5$ as primers.

Chromosome Preparations and In Situ Hybridization

Metaphase chromosome preparations from *T. nigerrimum* were obtained as described (Palomeque et al. 1988). Slides were denatured with the hybridization solution (20 ng probe/μl 50% formamide) for 2 min at 80°C and incubated in a moist chamber at 37°C overnight. After hybridization they were rinsed three times in 50% formamide at 37°C. Hybridization signals were detected using an avidin-alkaline phosphatase/biotinilated antiavidin system. Three rounds of amplification were performed using 100 μl of avidin-alkaline phosphatase (5 μg/ml) or biotinilated antiavidin (5 μg/ml) and finally an alkaline phosphatase DIG-detection kit (Roche). The chromosomes were subsequently stained with Giemsa.

Results and Discussion

Southern hybridization was used to detect telomeric sequences ($(TTAGG)_6$ and $(TTAGGG)_5$) in genomic DNA from *T. nigerrimum, D. melanogaster,* and the mouse (Figure 1). Using the $(TTAGGG)_5$ oligonucleotide as probe, under conditions of low stringency (final wash in 2× SSC at 55°C), strong hybridization could be observed in mouse and *T. nigerrimum,* while weak hy-

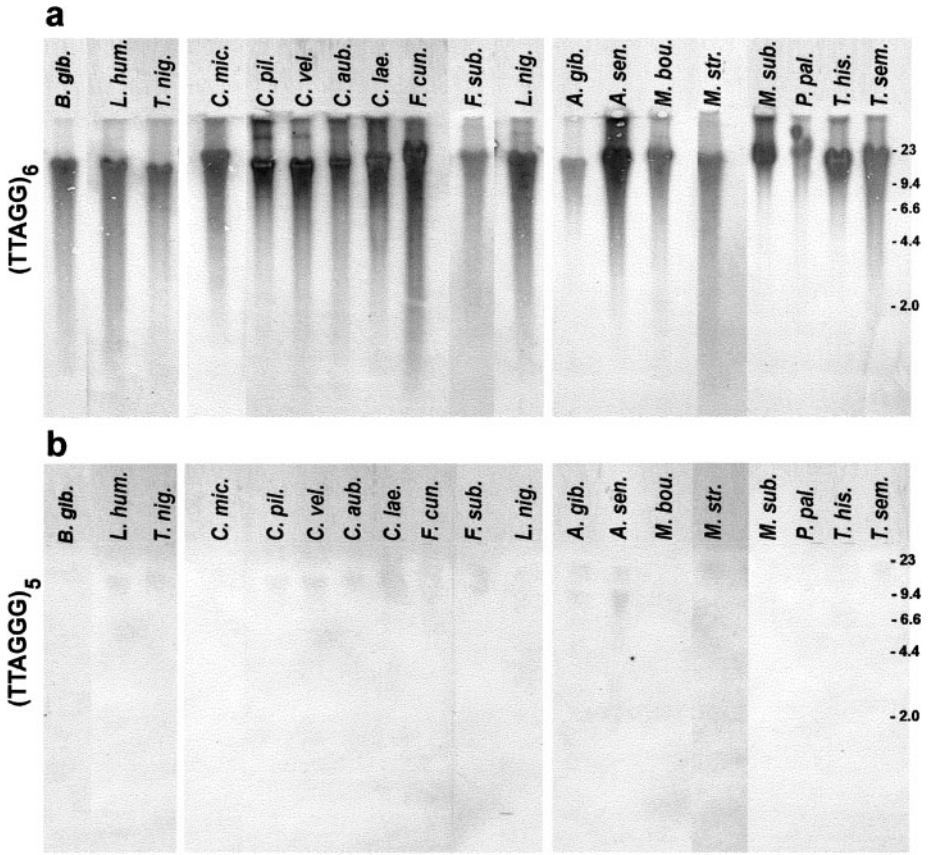

Figure 3. Southern hybridization to *Eco*RI-digested DNAs from 19 species of ants with **(a)** $(TTAGG)_6$ and **(b)** $(TTAGGG)_5$. Hybridization and wash conditions are described in Figure 2b. The species used were *Bothriomyrmex gibbus, Linepithema humile, Tapinoma nigerrimum* (subfamily Dolichoderinae), *Camponotus micans, Camponotus pilicornix, Cataglypis velox, Crematogaster auberti, Crematogaster laestrygon, Formica cunicularia, Formica subrufa, Lasius niger* (Subfamily Formicinae), *Aphaenogaster gibbosa, Aphaenogaster senilis, Messor bouvieri, Messor structor, Monomorium subopacum, Pheidole pallidula, Tetramorium hispanicum,* and *Tetramorium semilaeve* (subfamily Myrmicinae). Molecular weight ladder is in kb.

bridization was observed in *D. melanogaster* (Figure 1a). However, under high stringency (0.5× SSC or 0.1× SSC at 55°C), hybridization disappeared from *D. melanogaster* and *T. nigerrimum,* whereas in mouse the intense signal remained (Figure 1b). When $(TTAGG)_6$ was used as probe, hybridization signal was observed only in *T. nigerrimum* (Figure 1c).

The chromosome number of *T. nigerrimum* is $n = 9$ in males and $2n = 18$ in females and workers, and the standard haploid karyotype formula is $5m + 2sm + 2st$ (Palomeque et al. 1988). In situ hybridization to metaphase chromosomes was performed using TTAGGG- or TTAGG-repeat PCR-generated probes. No hybridization signals were found when TTAGGG repeat was used (data not shown). However, with the TTAGG repeat, strong signals were observed at the telomeres of the chromosomes (Figure 2). As can be seen in Figures 2b and c, there were some differences in hybridization intensities among different chromosomes and between homologous telomeres; probably due to technical artifacts, since these differences were not consistently observed when several metaphases were analyzed.

To determinate if other ant species contain $(TTAGG)_n$ in telomeric regions, DNAs from several species were digested with *Eco*RI and hybridized with the $(TTAGG)_6$ and the $(TTAGGG)_5$ probes under highly stringent conditions (0.5× SSC at 55°C). Figure 3a shows that all species tested presented intense hybridization signals when $(TTAGG)_6$ was used as a probe. However, when $(TTAGGG)_5$ was used as a probe, no clear hybridization signals were observed in any species, although a very faint band was noted in almost all species (Figure 3b).

The few species of Hymenoptera for which telomere organization has been studied have shown two types of patterns. Okazaki et al. (1993) performed Southern hybridization with two oligonucleotides, $(TTAGG)_6$ and $(TTAGGG)_5$, in the ant *M. yessensis,* and only with the TTAGG repeat was hybridization detected. Similar results were obtained with the honeybee (*A. mellifera*) (Sahara et al. 1999). However, Meyne et al. (1995) observed that telomeres of several species of ants (genus *Myrmecia*) hybridized with the putative insect telomere repeat sequence (TTAGG) and also with the putative vertebrate telomere sequences $(TTAGGG)_n$.

The presence of telomeric repeats in nontelomeric locations has been detected in a variety of species, generally associated with fusion-fission processes (Go et al. 2000; Slijepcevic 1998). We did not find any hybridization signals in nontelomeric locations. However, previous articles have reported that Robertsonian processes could play an important role in karyotypic evolution in the genus *Tapinoma* (Lorite et al. 1998b; Palomeque et al. 1988), as well as in other genera of the family Formicidae (Imai et al. 1984; Lorite et al. 1998a, 2000). The lack of nontelomeric hybridization could be due to processes of partial or total elimination after Robertsonian fission/fusion, resulting in no or very low copies of telomeric repeats in nontelomeric regions. The loss of telomeric repeats during Robertsonian processes has also been observed in other species (Silva and Yonenaga-Yassuda 1997).

The results obtained showed that the major telomere sequence of ants is TTAGG. In spite of this, TTAGGG repeats as a minor component in ant telomeric and subtelomeric regions could not be totally excluded. We did not detect positive signals by in situ hybridization using the $(TTAGGG)_n$ probe, although faint bands were observed by Southern hybridization. It is possible that the TTAGGG repeats are present in very low copy numbers and are consequently difficult to detect by in situ hybridization or Southern blotting techniques.

In summary, the presence of the pentanucleotide repeat TTAGG has now been found in species belonging to two families of Hymenoptera, the honeybee (*A. mellifera,* family Apidae) (Sahara et al. 1999), and in several species of ants (family Formicidae) belonging to the subfamilies Myrmeciinae (Meyne et al. 1995), Myrmicinae (Okazaki et al. 1993; present study), Dolichoderinae, and Formicinae (present study). Approximately 80% of all ant species belong to these four subfamilies (Bolton 1995), thus the TTAGG repeat may be a widespread telomere motif among the vast majority of ant species. However, the Hymenoptera (ants, bees, and wasps) is one of the most species-rich insect orders, with more than 200,000 described species (Bolton 1995), and further studies will be

needed to determinate the organization of telomeres in this insect order.

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Caudal Spotting in the Beacon Fish (*Hemigrammus ocellifer* Characidae)

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The beacon fish (*Hemigrammus ocellifer*) exhibits two phenotypes associated with spotting at the base of the caudal fin, with fish either possessing (*H. o. ocellifer*) or lacking (*H. o. falsus*) a prominent red spot in this region. Segregation patterns observed from the progenies of 15 different crosses support a hypothesis that caudal spotting in this species is controlled by a single gene with two alleles, for which the caudal spotting allele is completely dominant.

Fishes comprising the Characidae and several other closely related teleost families make up the traditional characins, a group of about 200 African and 1,000 South, Central, and southern North American species (Mills and Vevers 1982). Fishes in the genus *Hemigrammus* (Characidae) are particularly popular with aquarium hobbyists since they not only exhibit a wide variety of attractive coloration and marking patterns, but also are easily maintained and bred. The latter makes them ideally suited to environmental, behavioral, and genetic investigations (Brown et al. 1999, 2000; Buehrnheim and Fernandes 2001; Frankel 2000; Mikheev and Pakul'skaya 1989; Mikheev et al. 1992; Zhujkov and P'yanov 1993).

Within the species complex *Hemigrammus ocellifer* (beacon fish), two subspecies, *H. o. ocellifer* and *H. o. falsus,* are commonly recognized (Mills and Vevers 1982). Both subspecies are indigenous to northern South America. Phenotypically beacon fish are brown to greenish-yellow in color with a silvery iridescence. At the level of the dorsal fin, a dark transverse bar surrounded by striking golden-yellow spots characterizes the species and gives it its popular name. While both subspecies share these basic coloration and marking patterns, a brilliant red spot at the base of its caudal fin further distinguishes *H. o. ocellifer.* The inheritance of this spotting pattern is of particular interest, since it probably serves as an eyespot, mimicking the red color of the iris of the eye. As a result of recent investigations on the inheritance of coloration patterns in *Hemigrammus* (Frankel 2000) and on caudal peduncle banding in paradisefish (Frankel 2001), the present study was undertaken to ascertain the mode of inheritance of caudal spotting in the beacon fish.

Materials and Methods

Juvenile specimens of *H. o. ocellifer* and *H. o. falsus* were obtained from a local wholesale distributor in Virginia and maintained in separate 76 L holding tanks at 24°C. Male and female fish exhibiting the phenotypes were selected at random from stock specimens, placed in separate 76L tanks, and allowed to develop at 24°C until sexually mature. Optimal water conditions were provided for all fish (i.e., water hardness $\leq$ 5, pH 6.5, temperature 24°C) (Axelrod and Vorderwinkler 1995; Mills and Vevers 1982). All progeny for this study were obtained from artificial fertilizations as employed for the cyprinid fishes *Brachydanio rerio* and *B. albolineatus* (Frankel and Hart 1977; Hart and Messina 1972). Parentals exhibiting either the spotted (S) or unspotted (U) phenotype, along with F_1 progeny, were used in a series of 29 crosses (Table 1). Embryos from all crosses were incubated at 24°C in 250 ml fingerbowls containing tank water. Dead or developmentally arrested embryos were removed daily. Fry hatched 20–24 h after fertilization and were free swimming 48–72 h after hatching. Progeny groups were placed in separate 36 L rearing tanks, were fed initially on rotifers, and were allowed to develop until their phenotype could be visually determined. Phenotypic data of all progeny were recorded and subjected to chi-square analysis. Pooled and heterogeneity chi-square tests were also performed, treating the $U \times F_1$ and $F_1 \times F_1$ progenies as single large progenies in an analysis of overall goodness-of-fit.

Results and Discussion

Table 1 presents data for the proposed genotypes of parentals, and observed phenotypic numbers, expected ratios, and probability of fit for *H. ocellifer* analyzed for the mode of inheritance of caudal spotting for corresponding progeny groups. Parental fish and progeny from all crosses