Acrobeloides nanus is a dynamic colonizer nematode species, which exhibits high ecological plasticity. The purpose of this study was to investigate the genomic plasticity in A. nanus by examining the genomic divergence of a 70-kilodalton heat shock protein gene among different isolates of the species. Southern-blot hybridization experiments using as a probe the hsp70-1 sequences of Caenorhabditis elegans, resulted in two different hybridization patterns for the A. nanus isolates. Pattern I was similar to that of C. elegans. Pattern II showed two additional hybridization signals which apparently represent duplication(s) of the hsp70-1 locus in A. nanus. The A. nanus isolates were further differentiated by the variable size of the first intron of the hsp70-1 gene. This genomic variation in both neutral and under selection regions of the hsp70 genes reflects an extensive genomic plasticity in A. nanus, which could be correlated with the plasticity in ecology and adaptation of this opportunistic nematode species.

Key words: hsp70-1, Southern hybridization, PCR, intron length polymorphism, gene birth and death.

Abbreviations: Hsp70, heat shock protein 70 kilodalton; ER, endoplasmic reticulum; MT, mitochondria; CYT, cytoplasm; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
1990, 1999; Papatheodorou et al., 2004). Recently, different A. nanus populations and even different isolates were found to show high levels of genome plasticity, as exhibited by genomic divergence (intron length polymorphism) of the hsp70-3 gene (Nikolaïdis & Scouras, 2002).

The purpose of this study was to investigate the extent of genomic plasticity in A. nanus by examining the genomic divergence of a CYT Hsp70 gene among different isolates of the species. CYT hsp70s, due to their distinctive evolutionary pattern (see above), can be prominent molecular markers for the study of large-scale genomic events. In addition, the genomic differentiation of the hsp70s has been implicated in the adaptation and evolution of several organisms including nematodes (Cherkasova et al., 2000; Bettencourt & Feder, 2001; Michalak et al., 2001; Yokoyama et al., 2002).

**MATERIALS AND METHODS**

**Nematode specimens**

Nematodes were collected from Northern Greece (Petralona, Chalkidiki). All specimens reported here, were extracted from the same plot of soil (10 cm³) and cultivated as single worm lines. Eleven out of the 36 nematode lines were identified as A. nanus using morphological characters. Nematode cultures and DNA extraction were performed according to Nikolaïdis & Scouras (2002).

**Southern hybridization**

Three to four µg of genomic DNA were simultaneously digested with the restriction endonucleases BamH1, EcoRI, SalI (New England Biolabs), loaded onto a 1% agarose gel and transferred onto Hybond N+ membrane (Amersham Pharmacia Biotech). The probe used was the EcoRI - SalI fragment of C. elegans hsp70-1 (2.4 kb), which contains the 5’-untranslated region including the promoter sequences and almost the complete coding region of this gene (Fig. 1a; clone pCes401; kindly provided by D. L. Baillie). Hybridization was performed at 37°C in Denhardt’s solution containing 50% formamide, followed by medium stringency washes (3 × 15 min in 1 × SSC/0.5% SDS at 60°C), since the probe was heterologous. Plasmid DNA isolation, probe radiolabeling and autoradiography were performed as described by Konstantopoulou et al. (1998).

**PCR amplification**

The sequences of the primers used for the PCR amplification were 5’-CACCGAGCGTCTCATCG-3’ (forward) and 5’-CTCCGTGTCCCTCCTTG-3’ (reverse) targeting the genomic sequen-

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**FIG. 1.** (a) Genomic organization and restriction pattern of the hsp70-1 gene in Cae-

norhabditis elegans (clone pCes401; Snutch et al. 1988). Black boxes represent exons and white boxes introns. Arrows indicate the PCR primers (F, forward; R, reverse; see text and Fig. 2 for nucleotide sequences). E, EcoRI; B, BamHI; S, SalI. (b) Different hy-

bridization patterns observed among the Acr.obeloides nanus isolates. The probe used was the EcoRI - SalI fragment of C. elegans hsp70-1 (2.4 kb). Hybridization was per-

formed at 37°C in Denhardt’s solution containing 50% formamide, followed by medium stringency washes. Lanes: Ce, C. elegans; I, A. nanus isolates of Pattern I; II, A. nanus iso-

lates of Pattern II. Numbers indicate the ge-

nomic sizes in kilobases (kb).
cesses that include the first intron of hsp70-1 in C. elegans (Fig. 1a; Accession number M18540, F26D10.3). PCR amplification reactions were performed as in Nikolaidis & Scouras (2002), and the annealing temperature used was 48°C.

RESULTS

Southern hybridization

Two hybridization patterns of the hsp70-1 probe were identified among the A. nanus isolates (Fig. 1b). Pattern I, which was observed in 9 out of the 11 isolates, presented three strong hybridization signals, of approximately 2.4, 1.5, and 0.9 kb size, and is similar to the respective one of C. elegans (Fig. 1b). Pattern II shares the 2.4, 1.5, and 0.9 kb bands with pattern I and presents two additional strong hybridization signals of about 2.8 and 4.0 kb size (Fig. 1b). The weaker hybridization signals observed in C. elegans and A. nanus could represent a cross-hybridization of the hsp70-1 probe with other members of the Hsp70 multigene family, although their precise origin remains unclear.

The 2.4 kb band in C. elegans apparently represents the EcoRI - SalI fragment (hybridization probe), while the 1.5 and 0.9 kb bands correspond to EcoRI - BamHI, and Bam HI - SalI sub-fragments of the 2.4 kb band (Fig. 1a, b; see also Snutch et al., 1988). Therefore, the 2.4, 1.5 and 0.9 kb bands in all A. nanus isolates should represent the orthologous hsp70-1 gene in the latter species (Fig. 1b).

The high densities of the 2.8 and 4.0 kb hybridization signals in the A. nanus isolates of pattern II as well as the absence of other strong signals in C. elegans suggest that these additional bands also represent hsp70-1 related sequences rather than cross-hybridization with other hsp70s. In addition, the 2.8 and 4.0 kb bands probably represent paralogous sequences of the hsp70-1 gene in A. nanus rather than restriction fragment length polymorphism (RFLP). In the latter case, the isolates of pattern II should be heterozygous for one or more restriction sites of the hsp70-1 gene, a hypothesis that could explain only one additional hybridization signal. Therefore, we suggest that at least one of the 2.8 and 4.0 kb bands represents a duplication of the hsp70-1 locus in the A. nanus isolates of pattern II.

PCR amplification

After PCR amplification, four of the A. nanus isolates of pattern I presented a single band of approxi-

![Image](369x606 to 483x721)

FIG. 2. PCR amplification products including the first intron of the hsp70-1 gene for different Acrobeloides nanus isolates. The sequences of the primers used for the PCR amplification were 5’-CACCAGGATCTCATCG-3’ and 5’-CTCCGTGTCCTTCTTTG-3’ (forward and reverse, respectively) and the annealing temperature used was 48°C. Lanes: 1 = C. elegans, 2 = A. nanus isolates presenting the 500 bp band, 3 = A. nanus isolates presenting the 600 bp band. Numbers on the left indicate the PCR product sizes in base pairs (bp).

imately 500 bp, similar to that of C. elegans (Fig. 2). This band corresponds to the expected size of DNA fragments containing the first intron and its flanking sequences of the hsp70-1 gene in the latter species (Fig. 1a). The remaining A. nanus isolates of pattern I and the two isolates that exhibited pattern II (Fig. 1b) presented a single band of almost 600 bp (Fig. 2). This polymorphism, which is apparently the result of variable intron length, differentiates further the A. nanus isolates. Genomic divergence, due to different intron lengths, has previously been reported for the hsp70-3 gene in two A. nanus isolates (Nikolaidis & Scouras, 2002).

DISCUSSION

Our results show a substantial genetic differentiation associated with the hsp70-1 gene among the A. nanus isolates, and also suggest that hsp70-1 may be duplicated in two of the isolates. Hsp70-1 in C. elegans is located in a highly divergent genomic region (Snutch & Baillie, 1984). This gene has been duplicated in C. elegans resulting in the truncated pseudogene hsp-2 (reviewed by Heschl & Baillie, 1990). Caenorhabditis briggsae does not contain a sequence orthologous to the C. elegans pseudogene, as a result of the different rates of gene birth and death between the two closely related nematodes. The putative gene duplication in A. nanus suggests different rates of gene birth and gene death among isolates of the same
population. To our knowledge, this is the first time that such a case is described in the relevant literature for a metazoan species. Whether the paralogous sequences of hsp70-1 in A. nanus are functional or represent pseudogenes, as in the case of C. elegans, requires further investigation.

Irrespective of the functionality of the additional hsp70 copies, our results suggest extensive genome plasticity in A. nanus. Genome plasticity has been mainly reported for pathogenic bacteria and plants (Dorbrindt & Hacker, 2001; Ceccarelli et al., 2002). In these organisms genome plasticity is reflected by genomic alterations, frequent acquisition of DNA and loss of genetic information between domains that mainly contain repetitive DNA and allows rapid adaptation to environmental changes (Dorbrindt & Hacker, 2001; Rocha & Blanchard, 2002). Genomic divergence reflecting a substantial genomic plasticity has been described for microsatellite markers and the promoter of a highly heat-inducible Hsp70 gene between two D. melanogaster populations occupying different ecological niches (Michalak et al., 2001). Acrobeloides nanus represents a unique case, since different isolates from the same sampling plot exhibited both putative neutral (intron length) and under selection (gene number) genomic plasticity.

The genomic variation in Hsp70 genes could explain the extraordinary plasticity in ecology and adaptation, which allows species, such as A. nanus, to survive in a wide range of eco-geographical conditions. Further studies are essential to determine how genomic plasticity in the hsp70s is correlated with the ecological plasticity of the r-strategist nematode species A. nanus.

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