Chromosome Science **6** : 63-66, 2002

Brief Reports

A cytogenetic study of the common sole, *Solea solea*, from the Northern Adriatic Sea

A. Libertini[1], M. Mandrioli[2], M. S. Colomba[3], D. Bertotto[1], A. Francescon[1] and R. Vitturi[4]

[1]CNR Istituto di Scienze Marine, Riva 7 Martiri 1364/A, 30122-Venezia, Italy
[2]Dipartimento di Biologia Animale, Università di Modena e Reggio Emilia, Modena, Italy
[3]Facoltà di Scienze Ambientali, Università di Urbino, Urbino (PU), Italy
[4]Dipartimento di Biologia Animale, Università di Palermo, Palermo, Italy

Received March 11, 2003/Accepted August 1, 2003

Abstract. A cytogenetic study was carried out on the chromosomes and the nuclear DNA contents of a North-Western Mediterranean population of the common sole, *Solea solea* (Pisces, Pleuronectiformes, Soleidae). The chromosomes were studied using Giemsa-staining, C-banding, silver-staining and fluorescent *in situ* hybridization with 28S rDNA and 5S rDNA probes. The karyotype consisted of nine bi-armed (m, sm and st) and 12 mono-armed chromosome pairs (2n=42, NF=58). Heterochromatin blocks were located in the pericentromeric region of almost all of the chromosomes and along the entire short-arm of one sm-st pair, corresponding to the NOR site. The NORs showed size variation at individual level. Major (28S) and minor (5S) rDNA genes were located on different chromosome pairs. The diploid (2C) nuclear DNA content was 1.48±0.03 pg and the AT DNA corresponded to 59.22 % of the whole genome.

Keywords: Chromosomes, FISH, Nuclear DNA content, Common sole, Northern Adriatic Sea

Introduction

Thus far six species of the family Soleidae (Pleuronectiformes) have been cytogenetically studied. Chromosome number (2n) and fundamental number (NF) were reported for all of them (Klinkardt *et al.*, 1995; Vitturi *et al.*, 1993; Pardo *et al.*, 2001). Conventional banding techniques were attempted for three species (Vitturi *et al.*, 1993; Pardo *et al.*, 2001), and fluorochrome banding and the detection of major (18S and 28S) ribosomal genes (rDNAs) by *in situ* hybridization (ISH) were applied to only two species. In the literature there is no data on the nuclear DNA content in Soleidae (Gregory, 2002).

In regards to the common sole, *Solea solea* (L.), the chromosome number 42 was previously reported by Barker (1972) and Pardo *et al.* (2001). The latter investigated the karyotype and the location of major rDNAs by Giemsa, silver nitrate and CMA_3 staining, and ISH with a 18S rDNA probe in a North-Eastern Atlantic population (Galicia, Spain) of this species (Pardo, personal communication).

This paper reports the results of a cytogenetic study on a North-Western Mediterranean population of *S. solea*. Chromosomes were analyzed by Giemsa- and Ag-staining, C-banding, and fluorescence *in situ* hybridization (FISH) with 28S and 5S rDNAs as probes. The genome size and the AT nuclear DNA content was determined by flow cytometry.

Correspondence: Angelo Libertini.
e-mail: angelo.libertini@ismar.cnr.it

Materials and methods

Twenty-seven sexually mature specimens (ten males and 17 females) of *S. solea* (Pleuronectiformes: Soleidae) collected in the Gulf of Venice (North Adriatic Sea, North-Western Mediterranean) were used for this study.

Mitotic chromosome preparations were obtained from cephalic kidney cell suspension by means of the standard air-drying technique (Vitturi *et al.*, 1984). The chromo-

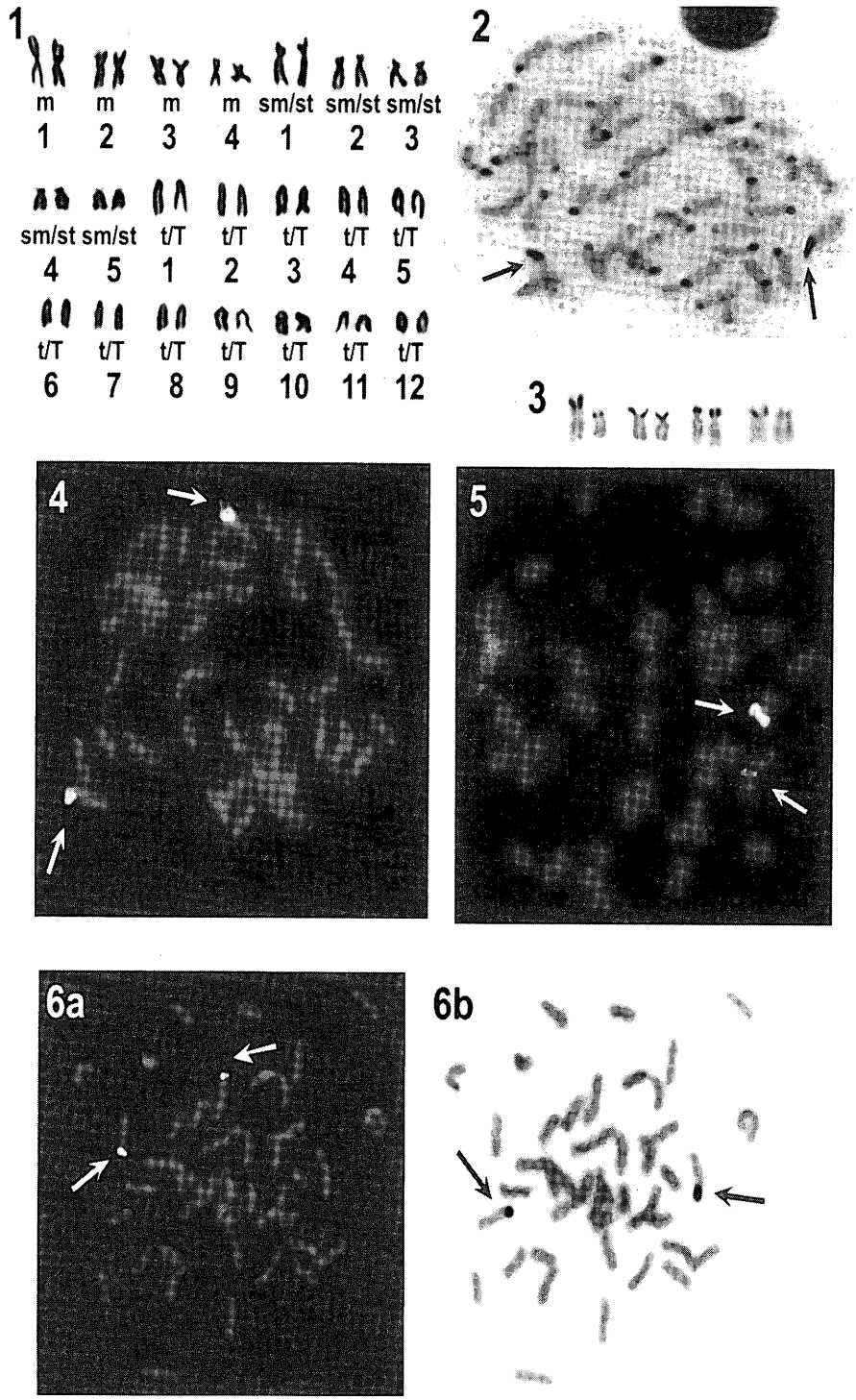

Figure 1-6. Metaphase chromosomes.

1. Giemsa stained karyotype of *Solea solea*. m=metacentric; sm/st=submetacentric/subtelocentric; t/T=acrocentric/telocentric.

2. The arrows indicate a large block of heterochromatin in the short arm of a chromosome pair after C-banding.

3. NOR-bearing chromosome pairs after staining with silver nitrate from plates of four different individuals.

4-5. FISH with 28S rDNA probe indicates polymorphic 28S rDNA signals (arrows).

6a. FISH with 5S rDNA probe indicates chromosomes with 5S rDNA signals (arrows).

6b. Sequential staining with silver nitrate indicates NOR-bearing chromosomes (arrows) of the same plate as in 6a.

some classification and abbreviations used were according to Levan *et al.* (1964), in that subtelocentrics were considered bi-armed.

Heterochromatin was revealed by C-banding according to Sumner (1972).

The active nucleolus organizer regions (NORs were detected by silver staining (Ag-NORs), following the method of Howell and Black (1980).

In order to map the 28S and 5S rDNA loci, homologous rDNA probes were obtained by PCR amplification according to Mandrioli *et al.* (2000).

Probes were DIG-labeled according to the protocol of the manufacturer. FISH-treated slides 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).

The genome size (GS) and Adenine-Thymine base-pair DNA content (AT-DNA) of the nuclei of disrupted kidney was measured by flow cytometry. Peripheral blood erythrocytes from chicken (GS=2.54 pg, AT-DNA=1.39 pg; Ronchetti *et al.*, 1995) were added to the kidney cell suspensions as an internal standard. The Nuclei were stained with propidium iodide (27 samples) and DAPI (ten samples) for GS and AT-DNA evaluation, respectively. For each sample at least 5,000 cells were analyzed, and the DNA index (mean channel number of the G1/G0 peak of the sole cells over the mean channel number of the G1/G0 peak of the chicken cells) was evaluated after elaboration of the fluorescence data by means of the Modfit software (Verity Software House). The average DNA indices among the analyzed samples, multiplied by the DNA content of the standard, gave the values assigned to the investigated species (data are reported as mean$\pm$standard deviation).

Results and discussion

All 27 specimens of *S. solea* from the Gulf of Venice had a diploid chromosome number of 2n=42. Their karyotype consisted of 21 chromosome pairs with four pairs of metacentrics (pairs m1-m4, in Fig. 1), five pairs of submeta-subtelocentrics (pairs sm/st1-sm/st5, in Fig. 1), and 12 pairs of acro-telocentric (pairs t/T1-t/T12, in Fig. 1). Therefore, the fundamental number of chromosome arms (NF) was 58. The present results were in agreement with those reported by Pardo *et al.* (2001) for a Western Atlantic population of the same species.

After C-banding (Fig. 2), heterochromatin was identified in the pericentromeric region of almost all of the chromosomes and along the entire short arms of a sm-st pair (Fig. 2, arrows).

Staining with silver nitrate localized the NOR on the whole short arm of a single chromosome pair (Fig. 3). Ag-NORs were characterized by length polymorphism and, therefore, the centromere position of the NOR bearing chromosomes could vary from sub-median to sub-terminal in the metaphase plates from different individuals (Fig. 3).

NOR localization and NOR polymorphism was confirmed by FISH with the 28S rDNA probe. Among the five specimens examined, two showed nearly identical size and intensity of hybridization signals (Fig. 4), while the signals differed in size (Fig. 5) in the remaining three specimens. FISH with the 5S rDNA probe was also carried out on five specimens, and revealed two positive sites per diploid genome, terminally located on a medium-sized chromosome pair (Fig. 6a). Silver-staining sequentially applied on the same metaphase plate (Figs. 6a and 6b) clearly indicated that 5S and 28S rDNA loci were located on different chromosome pairs. Different localization of 18S-28S rDNA and 5S rDNA has typically been shown by the most of the fish species investigated thus far (Inafuku *et al.*, 2000).

In *S. solea*, the NOR was a heterochromatic segment rich in GC base pairs, occupying the entire short-arm of a single chromosome pair, as revealed by *in situ* hybridization with major rDNA probes (18S and 21S), Ag-staining, C-banding and CMA_3-staining (Pardo *et al.*, 2001; present paper). The NORs are most likely structurally polymorph because of the differing numbers of repeated copies of the major rDNA gene cluster. Two distinct geographical populations of the common sole showed similar level of NOR polymorphism (Pardo *et al.*, 2001; present paper).

The mean (2C) value of GS and AT-DNA of *S. solea* were 1.48$\pm$0.03 pg and 0.86$\pm$0.01, respectively. This paper appears to be the first report of nuclear DNA contents within the family Soleidae (Gregory, 2002). In comparison with other species of Pleuronectiformes (Hinegardner and Rosen, 1972), the GS value of the common sole is intermediate within the range of values so far determined (1.30-2.20 pg). The genome of the common sole is richer in AT than in GC nucleotides and the percentage of AT-DNA (59.22 % of the whole genome) is consistent with the value found in *Limanda aspera* (59.0%), the only other flatfish analyzed for this parameter so far (Hudson *et al.*, 1980).

Acknowledgements

This study was supported by the Italian "Ministero per le Politiche Agricole e Forestali" in the frame of the 6th "Piano Triennale per la Pesca e l'Acquacoltura". The authors are deeply grateful to Miss Jennifer Vanecek for the English revision of the text.

References

Barker, C.J. (1972). A method for the display of chromosomes

of plaice, *Pleuronectes platessa*, and other marine fishes. Copeia **1972**: 365-368.

Gregory, T.R. (2002). Animal Genome Size Database. http://www.genomesize.com

Hinegardner, R. and Rosen, D.E. (1972). Cellular DNA content and the evolution of Teleostean Fishes. Amer. Natur. **106**: 621-644.

Howell, W.M. and Black, D.A. (1980). Controlled silver-staining of nucleolar organizer region with protective colloidal developer: a 1 step method. Experentia **36**: 1014-1015.

Hudson, P., Cuny, G., Cortadas, J., Haschemeyer, A.E.V. and Bernardi, G. (1980). An analysis of fish genomes by density gradient centrifugation. Eur. J. Biochem. **112**: 203-210.

Inafuku, J., Nabeyama, M., Kikuma, Y., Saitoh, J., Kubota, S. and Kohno, S. (2000). Chromosomal location and nucleotide sequences of 5S ribosomal DNA of two cyprinid species (Osteichthyes, Pisces). Chrom. Res. **8**: 193-199.

Klinkhardt, M., Tesche, M. and Greven, H. (1995). Database of Fish Chromosomes. Westharp Wissenschaften. Magdeburg, Germany, p. 237

Levan A., Fredga, K. and Sandberg, A.A. (1964). Nomenclature for centromeric position on chromosome. Hereditas **52**: 201-220.

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

Pardo, B.G., Bouza, C., Castro, J., Martinez, P. and Sánchez, L. (2001). Localization of ribosomal genes in Pleuronectiformes using Ag-, CMA_3-banding and *in situ* hybridization. Heredity **86**: 531-536.

Ronchetti, E., Salvadori, S. and Deiana, A.M. (1995). Genome size and AT-content in Anguilliformes. Eur. J. Histochem. **39**: 259-264.

Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. **75**: 304-306.

Vitturi, R., Carbone, P., Catalano, E. and Macaluso, M. (1984). Chromosome polymorphism in *Gobius paganellus* Linneo 1758 (Pisces, Gobiidae). Biol. Bull. **167**: 658-668.

Vitturi, R., Catalano, E. and Schillaci, A. (1993). Karyotypic characterization of 16 *Microchirus ocellatus* (L.) specimens (Pisces, Soleidae) using conventional and silver staining (NORs). Caryologia **46**: 41-45.