 *Cytologia* **69**(2): 175–179, 2004

# Chromosome Localization of the Ribosomal Genes 18S and 5S in Four Stocks of Rainbow Trout (*Oncorhynchus mykiss*) from Cultivated and Naturalized Stocks in Brazil

Fábio Porto-Foresti[1,*], Claudio Oliveira[2], Yara Aiko Tabata[3], Marcos Guilherme Rigolino[3] and Fausto Foresti[2]

[1] Departamento Ciências Biológicas, Faculdade de Ciências, Universidade Estadual Paulista (UNESP), Campus de Bauru, 17033–360, Bauru, SP, Brazil
[2] Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Campus de Botucatu, 18618–000, Botucatu, SP, Brazil
[3] Estação Experimental de Salmonicultura, Instituto de Pesca, 12460–000, Campos do Jordão, SP, Brazil

*Received February 4, 2004; accepted February 21, 2004*

**Summary** In the present study, fluorescence *in situ* hybridization (FISH) was employed to determine the chromosomal location of genes 18S rDNA and 5S rDNA in four rainbow trout stocks. In specimens from the stocks of Núcleo Experimental de Salmonicultura de Campos do Jordão and Gavião river, 18S genes were located at a subterminal position in the long arms of two submetacentric chromosomes, whereas in specimens from stocks of Mount Shasta and Teresópolis they were found in the short arms. In all analyzed stocks, 5S genes were located in two chromosome pairs. In a subtelocentric pair, 5S genes were present in the short arms and, in the other submetacentric pair, 5S genes were at an interstitial position. In the latter, 18S and 5S genes were contiguous. Taking into account that both 18S and 5S rDNA genes have been localized in the short arm of a submetacentric chromosome in almost all rainbow trout samples so far studied, the presence of such genes in the long arm, as seen in the samples from Núcleo Experimental de Salmonicultura de Campos do Jordão and Gavião river, supports the hypothesis of a pericentric inversion involving this chromosome segment in the ancestor line of these stocks. The observed polymorphism allowed the identification of a very useful genomic marker, and may therefore constitute an important tool in the genetic management of rainbow trout stocks.

**Key words** Fish cytogenetics, rDNA genes, Rainbow trout.

Fluorescence *in situ* hybridization (FISH) with ribosomal probes has been frequently used to locate nucleolar organizer regions (NORs) in fish chromosomes. To date, the information gathered generally confirms, and complements the results obtained by other techniques (Pendás *et al.* 1993a, Reed and Phillips 1995, Castro *et al.* 1997, Rossi *et al.* 1997). Fujiwara *et al.* (1998) reported that in the rainbow trout, *Oncorhynchus mykiss*, one chromosome pair contained the major rDNA genes (18S+5.8S+28S) and two chromosome pairs presented the minor rDNA genes (5S). Both major and minor genes were observed to be contiguously arranged in one of these chromosome pairs.

In the Atlantic salmon, *Salmo salar*, the FISH technique was used to show that probes for the major rDNA genes hybridize with the whole heterochromatic arms of the chromosomes bearing a secondary constriction. Previous studies using the Ag-NOR staining technique, suggested that NORs were restricted to that secondary constriction (Pendás *et al.* 1993a). Genes for the major rDNA were mapped in the brown trout (*Salmo trutta*) chromosomes using the FISH technique and indicate the presence of a main NOR-bearing chromosome, confirming the results obtained with the Ag-NOR staining technique (Pendás *et al.* 1993b). This study also showed the presence of eight

* Corresponding author, e-mail: fpforesti@fc.unesp.br

other chromosome pairs bearing minor NORs. The authors discussed the presence of those smaller NORs in heterochromatic regions and concluded that the results obtained by using fluorochromes, such as the chromomycin $A_3$ (widely used to identify active and inactive NORs in fish), should be interpreted with caution for this fluorochrome failed to identify the smaller NORs which were identified by the FISH technique.

The rainbow trout is an intensively reared species, with artificial populations worldwide spread, except in Antarctica (Hershberger 1992). Natural populations are found in western North America, from southern Alaska to southern Oregon and California. This and some other species of salmonids have been successfully introduced in Africa, South America, Australia and New Zealand (MacCrimmon 1971). In Brazil, the rainbow trout has been extensively cultivated since its introduction in 1949 (Faria 1953), from stocks imported at different times from different suppliers in the United States of America and other countries.

In this study, the fluorescence *in situ* hybridization technique was used to determine the chromosomal localization of the 18S and 5S rDNA genes in specimens from four rainbow trout stocks introduced in Brazil.

## Materials and methods

Three samples of rainbow trout (*Oncorhynchus mykiss*), obtained from cultivated stocks and one sample composed of individuals captured in the wild (naturalized) were studied (Table 1).

All specimens were submitted to cytogenetic analysis by the direct cell suspension method with kidney cells (Foresti *et al.* 1993). Before sacrifice, the animals were inoculated with a yeast cell suspension to increase the number of metaphase cells (Lozano *et al.* 1988). The procedure used to identify the nucleolar organizer regions (NORs) was that originally described by Howell and Black (1980). For FISH analysis, two rDNA sequences, 18S (about 1800 base pairs) and 5S (about 120 base pairs), isolated from *Oreochromis niloticus*, were used following the technique described by Porto-Foresti *et al.* (2002).

## Results and discussion

The identification of 18S rDNA genes by the FISH technique evidenced that the chromosomes of the specimens from Núcleo Experimental de Campos do Jordão presented either a single (N1 condition) or a double mark (N2 condition) at a subterminal position in the long arm of a submetacentric chromosome, enabling the identification of N1N1 and N1N2 phenotypes (Fig. 1a, b), as described by Oliveira *et al.* (1996) using the Ag-NOR staining technique. The specimens collected from the Gavião river, exhibited this same pattern of NORs distribution (Fig. 1c).

Individuals from the Mount Shasta and Teresópolis stocks, presented the 18S rDNA genes in

Table 1. List of the rainbow trout stocks used in the cytogenetic analysis and the identification of 18S and 5S rDNA genes

| Sampling site | Origin of sample[1] | Number of specimens | |
|---|---|---|---|
| | | Obtained | Analyzed |
| Núcleo Experimental de Salmonicultura de Campos do Jordão, SP | Campos do Jordão[2] | 30 | 10 |
| Gavião river | São José dos Barreiros | 15 | 6 |
| Salmonicultura N R, Sapucaí Mirim, MG | Mount Shasta | 40 | 10 |
| Aquacultivo Montenegro, Teresópolis, RJ | Teresópolis[2] | 20 | 10 |

[1] The origin of samples at each fish farm was certified by landowners and managers. [2] Stocks kept for more than 10 yr.

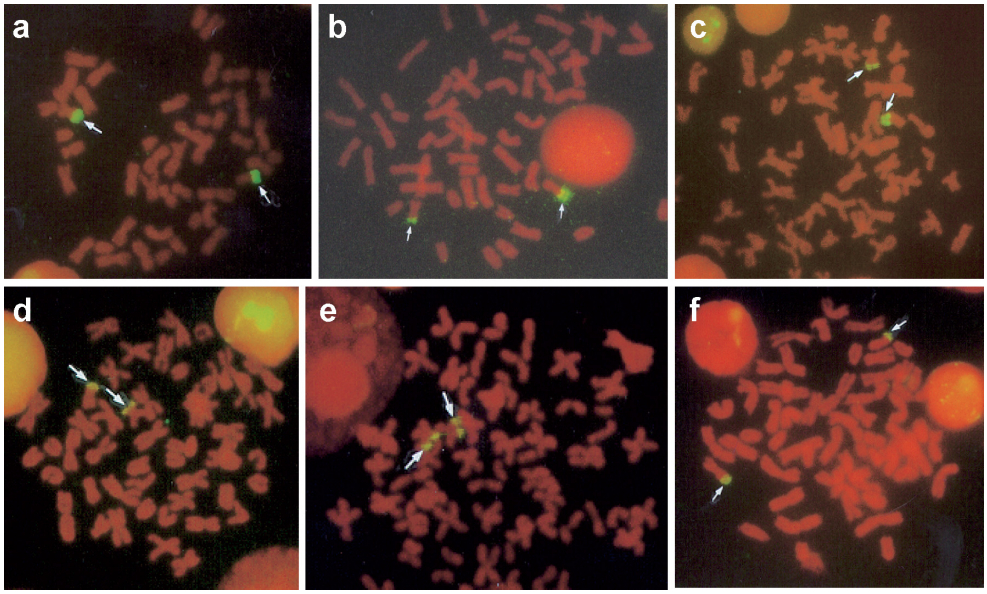


Fig. 1. Metaphases after rDNA 18S probe *in situ* hybridization of four samples of rainbow trout (*Oncorhynchus mykiss*) introduced in Brazil. a, b) Núcleo Experimental de Salmonicultura de Campos do Jordão, c) Gavião River, d) Mount Shasta, e, f) Teresópolis. Arrows indicate rDNA sites in one chromosome pair.

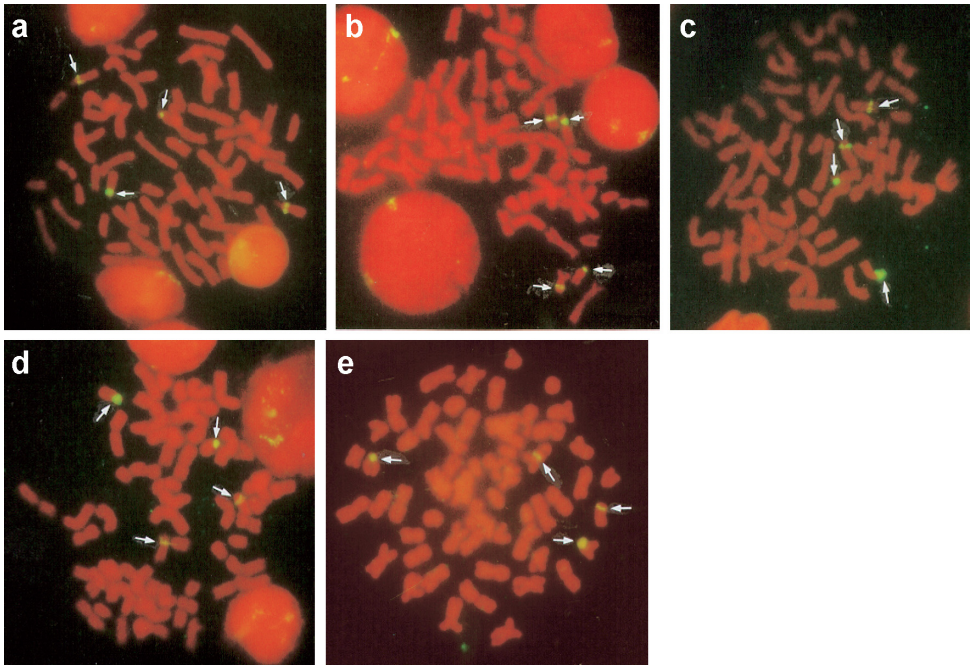


Fig. 2. Metaphases after rDNA 5S probe *in situ* hybridization of four samples of rainbow trout (*Oncorhynchus mykiss*) introduced in Brazil. a) Núcleo Experimental de Salmonicultura de Campos do Jordão, b) Gavião River, c) Mount Shasta, d, e) Teresópolis. Arrows indicate rDNA sites in two chromosome pairs.

the short arm of a submetacentric chromosome pair (Fig. 1d, e). However, in some specimens from Teresópolis, 18S rDNA genes were located in the short arm of a submetacentric chromosomes and in the long arm of other submetacentric chromosome (Fig. 1f). The particular NOR phenotypes observed in those individuals suggest the occurrence of hybridization between the individuals from a newly introduced stocks (Teresópolis) and those from the Campos do Jordão stock.

The results obtained with the 5S rDNA probes showed the occurrence of two chromosome pairs bearing these genes in all stocks analyzed, as described by Fujiwara *et al.* (1998). One 5S rDNA cluster was identified in the short arms of a subtelocentric chromosome pair and a second cluster was identified at an interstitial position in a submetacentric pair. In the latter, 18S and 5S genes were contiguously arranged as previously reported by Fujiwara *et al.* (1998).

In the subtelocentric chromosome pair, in which a mark was observed in the pericentromeric region, there were no detectable differences in the samples. On the other hand, in the specimens from Núcleo Experimental de Campos do Jordão and the Gavião river, signals were observed in the interstitial region on the long arm of a chromosome pair (Fig. 2a, b). In individuals from Mount Shasta and Teresópolis, marks were detected at a subterminal position in the short arm of a submetacentric chromosome pair (Fig. 2c, d). In some fish from Teresópolis, it was also observed that marks evidenced by the 5S rDNA probe were localized at different positions in a submetacentric chromosome pair. One of those marks was seen at a subterminal position in the short arm, while the other one was found at a subterminal position in the long arm (Fig. 2e).

The different NOR localizations observed suggest that a pericentric inversion took place and presumably involved the chromosome segment containing both 18S rDNA and 5S rDNA genes. The existence of a polymorphism in some specimens from the Teresópolis stock suggests the occurrence of hybridization between individuals from the original imported stock and specimens from Núcleo Experimental de Salmonicultura de Campos do Jordão.

The data obtained from the Gavião river specimens support the hypothesis that the stock employed during the introduction program conducted in 1962 with fish from California (MacCrimmon 1971) was also used to supply Núcleo Experimental de Salmonicultura de Campos do Jordão.

The polymorphism observed in the examined samples constitutes an important tool to be used as a genomic marker in this species. The technique showed to be useful either in the identification of both reared and naturalized stocks, and could be also used to monitor new imported stocks introduced in this region in the future.

## Acknowledgments

The authors are grateful to R. Devidé and A. L. Alves for technical assistance, to Mr. Afonso Martins from Salmonicultura N.R. and to Mr. Alberto Carvalho from Aquicultura Montenegro for the donation of part of the fish used in this study, to Dr. P. M. Galetti Jr., Dr. O. Moreira Filho, Dr. N. Castagnoli, Dr. J. F. Lui, for a critical review of the original manuscript. Funds supporting this study were provided by FAPESP.

## References

Castro, J., De Lucchini, S., Nardi, I., Sánchez, L. and Martinez, P. 1997. Molecular analysis of a NOR site polymorphism in brown trout (*Salmo trutta*): organization of rDNA intergenic spacers. Genome **40**: 916–922.

Faria, A. 1953. Dados sobre a biologia da truta arco-íris. Ministério da Agricultura-Departamento Nacional de Produção Animal Divisão de Caça e Pesca Press: Rio de Janeiro.

Foresti, F., Oliveira, C. and Almeida-Toledo, L. F. 1993. A method for chromosome preparations from large specimens of fishes using *in vitro* short treatment with colchicine. Experientia **49**: 810–813.

Fujiwara, A., Abe, S., Yamaha, E., Yamazaki, F. and Yoshida, M. C. 1998. Chromosomal localization and heterochromatin association of ribosomal RNA gene loci and silver-stained nucleolar organizer regions in salmonid fishes. Chro-

mosome Res. **6**: 463–471.

Hershberger, W. K. 1992. Genetic variability in rainbow trout populations. Aquaculture **100**: 51–71.

Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia **36**: 1014–1015.

Lozano, R., Rejon, C. R. and Rejon, M. R. 1988. A method for increasing the number of mitoses available for cytogenetic analysis in rainbow trout. Stain Technology **66**: 335–338.

MacCrimmon, H. R. 1971. World distribution of rainbow trout (*Salmo gairdneri*). J. Fish. Res. B. Can. **28**: 663–704.

Oliveira, C., Foresti, F., Rigolino, M. G. and Tabata, Y. A. 1996. Paracentric inversion involving a NOR-bearing chromosome of rainbow trout (*Oncorhynchus mykiss*): electron microscopy studies of the synaptonemal complex. Caryologia **49**: 335–342.

Pendás, A. M., Morán, P. and Garcia-Vazquez, E. 1993a. Ribosomal RNA genes are interspersed throughout a heterochromatic chromosome arm in Atlantic salmon. Cytogenet. Cell Genet. **63**: 128–130.

—, — and — 1993b. Multi-chromosomal location of ribosomal RNA genes and heterochromatin association in brown trout. Chromosome Res. **1**: 63–67.

Porto-Foresti, F., Oliveira, C., Tabata, Y. A., Rigolino, M. G. and Foresti, F. 2002. NORs inheritance analysis in crossings including individuals from two stocks of rainbow trout (*Oncorhynchus mykiss*). Hereditas **136**: 227–230.

Reed, K. M. and Phillips, R. B. 1995. Molecular cytogenetic analysis of the double-$CMA_3$ chromosome of lake trout, *Salvelinus namaycush*. Cytogenet. Cell Genet. **70**: 104–107.

Rossi, A. R., Gornung, E. and Crosetti, D. 1997. Cytogenetic analysis of *Liza ramada* (Pisces, Perciformes) by different staining techniques and fluorescent *in situ* hybridization. Heredity **79**: 83–87.