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Research Article

Analysis of NOR distribution in cultivated and naturalized stocks of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

In salmonid species, nucleolar organizing regions (NORs) have frequently been found on a single chromosome pair. However, some other, smaller yet active NORs have also been described. In the present study, NORs were found to be located in different positions on the chromosomes of 12 rainbow trout samples (11 cultivated and one naturalized stocks). Three phenotypes were found: 1) NORs located on two submetacentric chromosomes, in a subterminal position on the short arm (AA); 2) NORs located on two submetacentric chromosomes, in a subterminal position on the long arm (BB); and 3) NORs located on two submetacentric chromosomes, one in a subterminal position on the short arm, and the other in a subterminal position on the long arm (AB). Phenotype AA was identified in individuals from ten cultivated stocks, phenotype AB was identified in individuals from three cultivated stocks, and phenotype BB was found in one cultivated and one wild stock. Some aspects of the origin and the distribution of the different phenotypes are discussed.

Key words: NOR polymorphism, fish cytogenetics, rainbow trout.

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Introduction

Salmonid fish were tentatively introduced in Brazil in the beginning of the twentieth century. In 1962, a large official program was set up, and rainbow trout eggs were imported from California (EUA), in order to start the culture of this species in fish farms, and also to introduce it into some rivers of the Serra da Bocaina (Rio de Janeiro and São Paulo), Serra da Mantiqueira (São Paulo), and other mountain fluvial systems, located in the northern region of the state of Santa Catarina (MacCrimmon, 1971).

Cytogenetic studies have shown that salmonid species frequently have a single chromosome pair with active nucleolus organizer regions (NORs) (Phillips and Ihssen, 1985; Phillips *et al.*, 1986). However, additional NORs have been described in a few specimens (Phillips *et al.*, 1989; Pendás *et al.*, 1993). Fluorescence hybridization studies with 18S probes in the brown trout, *Salmo trutta*, revealed that, in addition to larger aggregations of rDNA in one chromosome pair, 16 other, smaller, transcriptionally inactive regions were also present (Pendás *et al.*, 1993).

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In the rainbow trout *Oncorhynchus mykiss*, several studies have shown that NORs are located in a subterminal position on the short arm of a submetacentric chromosome pair (Schmid *et al.*, 1982; Phillips and Ihssen, 1985; Mayr *et al.*, 1986; Ueda and Kobayashi, 1988; Lloyd and Thorgaard, 1988). Oliveira *et al.* (1996) observed, however, that rainbow trouts from *Núcleo Experimental de Salmonicultura de Campos do Jordão*, imported from California (USA) in the 1960's, exhibited NORs in a subterminal position on the long arm of a submetacentric chromosome pair (Table I).

In the present study, the Ag-NOR technique was used to determine and compare the chromosome location of the NORs in 11 rainbow trout samples obtained from cultivated and naturalized stocks in the states of São Paulo, Rio de Janeiro and Minas Gerais.

Material and Methods

Cytogenetic analysis was performed on rainbow trout (*Oncorhynchus mykiss*) samples from twelve different cultivated stocks and one sample captured in the wild (naturalized) (Table I). Some specimens of each stock were marked with magnetic tags and kept in tanks at Núcleo

Table I - List of the rainbow trout (*Oncorhynchus mykiss*) stocks employed for cytogenetic analyses and identification of NOR phenotypes.

Sampling site	Sample code	Sample origin[1]	Number of specimens		NOR phenotype[6]
			Obtained	Analyzed	
Núcleo Experimental de Salmonicultura de Campos do Jordão, São Paulo	Stock 1	California, USA[2]	-	19	BB
Núcleo Experimental de Salmonicultura de Campos do Jordão, São Paulo	Stock 2	Isle of Man, England	30	10	AA
Salmonicultura N R, Sapucaí Mirim, Minas Gerais	Stock 3	Mount Shasta, USA	40	10	AA
Salmonicultura N R, Sapucaí Mirim, Minas Gerais	Stock 4	Kamloops, Canada	30	10	AA
Núcleo Experimental de Salmonicultura de Campos do Jordão, São Paulo	Stock 5	Denmark[4]	50	10	AA
Aquacultivo Montenegro, Teresópolis, Rio de Janeiro	Stock 6	Teresópolis[3]	20	10	AA - AB
Salmonicultura Hotel Fazenda São João, Nova Friburgo, Rio de Janeiro	Stock 7	Nova Friburgo[3]	25	10	AA
Salmonicultura Hotel Fazenda São João, Nova Friburgo, Rio de Janeiro	Stock 8	USA	20	10	AA
Salmonicultura Hotel Fazenda São João, Nova Friburgo, Rio de Janeiro	Stock 9	Denmark[5]	30	10	AA
AQUA Ltda, Bananal, Rio de Janeiro	Stock 10	Serra da Bocaina[3]	25	8	AA - AB
Núcleo Experimental de Salmonicultura de Campos do Jordão, São Paulo	Stock 11	Japan	30	10	AA - AB
Gavião River, Bananal, São Paulo	Stock 12	São José dos Barreiros, SP	15	6	BB

[1]The origin of samples at each fish farm was certified by landowners and managers.
[2]Stock kept isolated in Campos do Jordão for over 30 years and studied by Oliveira *et al*. (1996).
[3]Stocks kept isolated for over 10 years.
[4]Stock recently (less than 5 years ago) imported by Associação Brasileira de Truticultores.
[5]Stock recently (less than 5 years ago) imported by the Hotel Fazenda São João.
[6]AA = NORs located in a subterminal position on the short arms of two submetacentric chromosomes; BB = NORs located in a subterminal position on the long arms of two submetacentric chromosomes; and AB = NORs located on two submetacentric chromosomes, one in a subterminal position on the short arm, and the other in a subterminal position on the long arm.

Experimental de Salmonicultura de Campos do Jordão, São Paulo, Brazil.

Direct suspensions of kidney cells (Foresti *et al.*, 1993) were used to analyze the chromosomal characteristics of the individuals, after prior injection of the fish with a yeast cell suspension, to increase the number of metaphase cells (Lozano *et al.*, 1988). The procedure used to identify NORs was originally described by Howell and Black (1980).

Results and Discussion

Studies including the identification of NORs have appointed them as important cytogenetic markers for aquaculture research (Fergunson *et al.*, 1995). Moreover, several investigations have demonstrated that polymorphic NOR phenotypes are inherited (Mikelsaar *et al.*, 1977; Markovic *et al.*, 1978; Henderson and Bruere, 1980; Arruda and Monteagudo, 1989).

In the present study, three NOR phenotypes were found: 1) NORs located in a subterminal position on the short arms of two submetacentric chromosomes (AA); 2) NORs located in a subterminal position on the long arms of two submetacentric chromosomes (BB); and 3) NORs located on two submetacentric chromosomes, one in a subterminal position on the short arm, and the other in a subterminal position on the long arm (AB).

The AA phenotype was the most common, and was found in specimens from ten cultivated stocks (Table I and Figures 1a-h, j, l). This observation is in accordance with most of data published on NOR distribution in rainbow trout (Schmid *et al*., 1982; Phillips and Ihssen, 1985; Mayr *et al.*, 1986; Ueda and Kobayashi, 1988; Lloyd and Thorgaard, 1988; Phillips *et al.*, 1989). Phenotype AB was observed in 20% of the specimens from stock 6, 20% from stock 10, and 30% from stock 11 (Table I and Figures 1i, k, m). This pattern has not been described in any previous study.

Taking into account that phenotype BB is a characteristic of the rainbow trouts from stock 1 (Oliveira *et al.*, 1996 - Table I, Figure 1n), the occurrence of phenotype AB in some individuals of stocks 6, 10, and 11 may be due to hy-

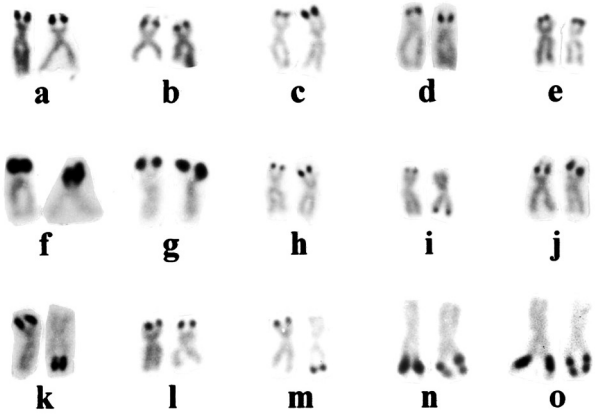

Figure 1 - Location of the nucleolar organizing regions (NORs) in samples of rainbow trout (*Oncorhynchus mykiss*) introduced in Brazil. a) Isle of Man (stock 2); b) Mount Shasta (stock 3); c) Kamloops (stock 4); d) Denmark (Associação Brasileira de Truticultores) (stock 5); e) Nova Friburgo (stock 7); f) USA (stock 8); g) Denmark (Hotel Fazenda São João) (stock 9); h) Teresópolis (stock 6); i) hybrid sample from Teresópolis (stock 6); j) Serra da Bocaina (stock 10); k) hybrid sample from Serra da Bocaina (stock 10); l) Japan (stock 11); m) hybrid sample from Japan (stock 11); n) California, USA (stock 1); o) Gavião river (stock 12).

bridization processes between fish from these stocks and fish from stock 1. Considering the managing process of the stocks involved, this was expected, because eggs and fries of the rainbow trouts from stock 1 have been widely distributed throughout trout farms in the states of São Paulo, Rio de Janeiro and Minas Gerais, where this species is cultivated.

Cytogenetic analysis revealed that all fish of stock 12 exhibited phenotype BB (Figure 1o). It was also observed that one of the submetacentric chromosomes had a double mark at the terminal portion of its long arm, similar to that found by Oliveira *et al.* (1996) in stock 1.

The presence of phenotype BB and of a characteristic chromosome with two NOR segments in the individuals from stock 1 (from Núcleo Experimental de Salmonicultura de Campos do Jordão) and stock 12 (Gavião river) suggests that both stocks have a common ancestor. This observation agrees with MacCrimmon (1971), who reported that the rainbow trout samples introduced in the mountain areas of São Paulo state came from California in the early 1960's. The study of other naturalized populations and the use of alternative molecular markers may lead to a better understanding of the relationships among rainbow trout stocks in Brazil.

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