CARYOLOGIA Vol. 38, n. 3-4: 319-324, 1985

NUCLEOLAR ORGANIZING REGIONS IN SOME SPECIES OF NEOTROPICAL CICHLID FISH (PISCES, PERCIFORMES)

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SUMMARY — In the present study, 10 neotropical species of family Cichlidae (Pisces, Perciformes) were submitted to analysis of the nucleolar organizing regions (NORs). In 8 of these species, the NORs were located in the first pair in the complement and coincided with the secondary constrictions observed there, whereas in *Cichlasoma facetum* and *Geophagus brasiliensis* the NORs were located in another chromosome pair. The possibility that the predominant location of NORs in the 1st pair in the karyotype indicates a more primitive condition of this group of fish is discussed. Variations in NOR size between homologous chromosomes of some species were also observed.

INTRODUCTION

The study of the nucleolar organizing regions (NORs) has been constantly growing, especially with the use of $AgNO_3$ staining. Most fish studied in terms of NORs have shown in general only one pair of homologous chromosomes stained with silver. However, cases showing more than one nucleolar organizing pair, as well as intra and interspecific and even intraindividual variations have been observed, as shown in the review by MOREIRA F° (1983).

Few reports have been published on the NORs of family Cichlidae. In *Sarotherodon galilaeus*, KORNFIELD *et al.* (1979) found two submetacentric nucleolar chromosomes, which were probably non-homologous and frequently occurred in association. FORESTI *et al.* (1983), in a study of the synaptonemic complex during male meiosis in *Tilapia rendalli*, detected the presence of 2 silver grain clusters on 2 bivalents, representing 4 chromosomes with active NORs.

Research carried out with the financial support of Programa Integrado de Genética (PIG) - CNPq and of the Federal University of São Carlos.

In view of these considerations, the present study was undertaken to investigate the nucleolar organizing regions in some neotropical species of Cichlidae in order to obtain additional information on the number, localization and behavior of these structures in this large fish family.

MATERIAL AND METHODS

Ten cichlid species belonging to 7 different genera were investigated (*Astronotus ocellatus, Batrachops semifasciatus, Cichlasoma facetum, Chaetobranchopsis australe, Crenicichla lepidota, Crenicichla lacustris, Crenicichla vittata, Geophagus brasiliensis, Geophagus surinamensis* and *Gymnogeophagus balzanii*). The specimens were collected in different localities in Brazil.

Kidney cell metaphases were prepared by the air-drying technique of EGOZCUE (1971), modified by BERTOLLO *et al.* (1978). The nucleolar organizing regions were studied by the silver nitrate staining method (GOODPASTURE and BLOOM 1975), with some modifications as in PATHAK and KIEFFER (1981).

RESULTS

In all of the species studied, one homologous chromosome pair showed nucleolar organizing regions, except for *Chaetobrachopsis australe*, in which only one chromosome showed NORs (*Fig. 1*). In three species (*Geophagus brasiliensis, Cichlasoma facetum* and *Crenichla lacustris*), variations in NOR size were also observed between the respective NOR-bearing homologous chromosomes (Fig. 1 C, E, H).

The karyotype of each of the 10 species studied is characterized by a chromosome number of $2n = 48$ (FELDBERG and BERTOLLO 1985). In 8 species, the NORs were located on the first pair in the complement, while in the remaining two (*Geophagus brasiliensis* and *Cichlasoma facetum*) they were located on a relatively large, not fully identifiable pair, which, however, was not the 1st pair in the karyotype (Fig. 1).

DISCUSSION

In most organisms, the NORs are visible as a secondary constriction in metaphase chromosomes (FERGUSON-SMITH 1964), even though these constrictions do not always possess rDNA and, conversely, rDNA is not always located in secondary constriction regions (RUIZ 1982).

According to WARBURTON and HENDERSON (1979), in organisms in which only one chromosome pair with a secondary constriction is observed, generally that is the pair in which the nucleolar organizing region is located, as was the

case for the species studied here. The NOR-bearing chromosomes were also relatively constant, corresponding to the first pair in 8 of the 10 species studied. However, even though NORs were predominantly located on this chromosomes, there was some variation in their position. Thus, in four species (*Astronotus ocellatus, Batrachops semifasciatus, Crenicichla lacustris* and *Crenicichla vittata*) the NORs occupied an interstitial position on the short arm, and

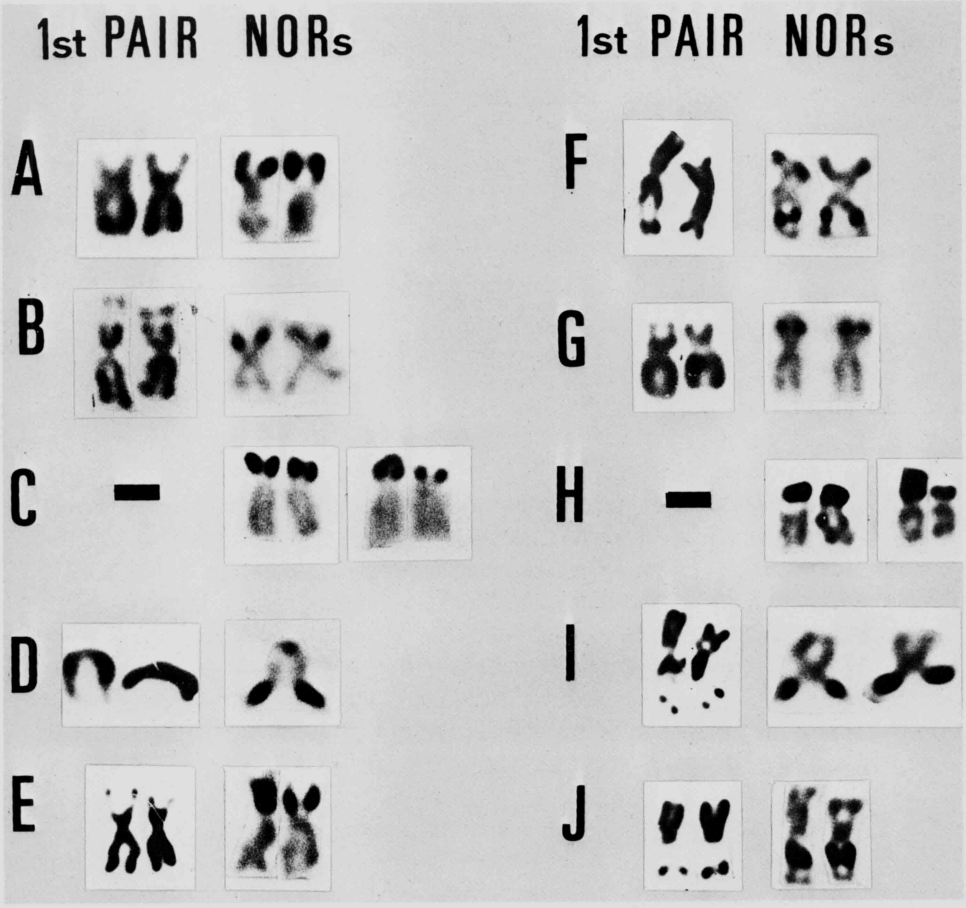

Fig. 1. — Nucleolar organizing regions (NORs) in the species studied. (A) *Astronotus ocellatus*, (B) *Batrachops semifasciatus*, (C) *Cichlasoma facetum*, (D) *Chaetobranchopsis australe*, (E) *Crenicichla lacustris*, (F) *Crenicichla lepidota*, (G) *Crenicichla vittata*, (H) *Geophagus brasiliensis*, (I) *Geophagus surinamensis* and (J) *Gymnogeophagus balzanii*. In each species, the nucleolar organizing pair corresponds to the first pair in the karyotype, which can be M-SM (meta-submetacentric) or ST-A (subtelo-acrocentric). Only in *Cichlasoma facetum* (C) and *Geophagus brasiliensis* (H) the nucleolar organizing pair was not fully identified one, which was not the first pair. In these two species, as well as in *Crenicichla lacustris* (E), an heteromorphism in the NOR size was observed in some metaphases.

TABLE 1 - *Location of the nucleolar organizing regions (NORs) in the species studied.*

Species	NORs location	
	Chromosomes	Position
Astronotus ocellatus	1° pair (M-SM)	interstitial - short arm
Batrachops semifasciatus	1° pair (M-SM)	interstitial - short arm
Cichlasoma facetum	not identified (ST-A)	terminal - short arm
Chaetobranchopsis australe	1° pair (ST-A)	terminal - long arm
Crenicichla lacustris	1° pair (M-SM)	interstitial - short arm
Crenicichla lepidota	1° pair (M-SM)	interstitial - long arm
Crenicichla vittata	1° pair (M-SM)	interstitial - short arm
Geophagus brasiliensis	not identified (ST-A)	terminal - short arm
Geophagus surinamensis	1° pair (M-SM)	interstitial - long arm
Gymnogeophagus balzanii	1° pair (M-SM)	interstitial - long arm

in three species (*Crenicichla lepidota, Geophagus surinamensis* and *Gymnogeophagus balzanii*) an interstitial position on the long arm of the first pair, which in these 7 species is of the M-SM type. *Chaetobranchopsis australe* had a pratically terminal NOR on the long arm of chromosome 1, which, however, in this case is of the ST-A type. In the two remaining species (*Geophagus brasiliensis* and *Cichlasoma facetum*), in which the nucleolar organizing region was not located in the first pair in the karyotype, location was terminal on the short arm of one of the chromosomes pairs ST-A (Table 1 and Fig. 1).

The number of nucleolar organizing chromosome and the NOR size may vary from species to species and even intraspecifically (GIANNONI *et al.* 1981; FORESTI *et al.* 1981; MORELLI 1981; RUIZ *et al.* 1981; SCHMID 1982). In the present study, the number of nucleolar chromosomes was always two, except for *Chaetobranchopsis australe* (Fig. 1-D), whose 2 specimens investigated exhibited a nucleolar organizing region in only one of the homologues. Coincidentally, only one of these chromosomes showed the nucleolar secondary constriction. The data obtained thus far, however, are not sufficient to permit us to determine whether this is a case of non-transcription of one of the NORs in the preceding metaphase or of deletion of this region, although the high incidence of metaphases with a single NOR and the visualization of a single secondary constriction may eventually favor this latter hypothesis.

In *Cichlasoma facetum, Crenicichla lacustris* and *Geophagus brasiliensis*, an heteromorphism may occur with respect to NOR size between the two homologues of the NOR-bearing pair (Fig. 1 C, E, H). This variation occurred both intra-and interindividually, with NORs of similar or different sizes being observed. No case of apparently increased NOR was observed in homozygosis. The data obtained may indicate both a difference in the amount of ribosomal cistrons between the NORs as a difference in the activity of these genes.

It is known that a variation in activation and/or cistron number may occur

between different NORs in the same individual. For example, the number of cistrons for ribosomal RNAs (18S and 28S) varies between different chromosomes and even between homologues (WARBURTON *et al.* 1976; WARBURTON and HENDERSON 1979). It is possible that the genic redundancy of rDNA may cause unequal permutations, duplications or spontaneous deletions leading to a variation in the amount of gene copies in the nucleolar organizing regions (RITOSSA *et al.* 1966). The tendency of nucleolar chromosomes to associate to form the nucleolus facilitates rDNA exchanges between the NORs, whether or not they are homologous, thus contributing to the variability in the number of ribosomal cistrons (RUIZ 1982).

The karyotypic evolution of neotropical Cichlidae indicates a more conservative than divergent scheme from the numerical point of view, with the majority of species thus far studied exhibiting $2n = 48$ chromosomes (THOMPSON 1979; FELDBERG and BERTOLLO 1985). In turn, the predominant localization of NORs in the 1st pair in the karyotype also appears to indicate another feature that may have been maintained in a more conservative manner throughout the karyotypic evolution of these fishes. If this is the case, this location of the nucleolar organizing regions may characterize a more primitive condition in this group, in such a way that the different positions of the NORs in the first pair in the karyotype or their location on another chromosome pair (Table 1) may be due to chromosomal rearrangements of the inversion and translocation type, respectively, involving the ribosomal cistrons.

However, since the neotropical species of Cichlidae are quite numerous, a wider cytogenetical study of their nucleolar organizing regions is needed to investigate with more assurance the behavior of these structures during the karyotypic evolution of this group as a whole.

Acknowledgements. — The authors are grateful to Dr. Heraldo A. BRITSKI, Museum of Zoology of the University of São Paulo, for identifying the species analysed, and to SUDEPE for authorizing the specimens capture.

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Received 16 October 1984; accepted 18 March 1985