

## KARYOTYPIC VARIABILITY IN *Hoplerythrinus unitaeniatus* (PISCES, CHARACIFORMES, ERYTHRINIDAE). II. OCCURRENCE OF NATURAL TRIPLOIDY

Lucia Giuliano-Caetano<sup>1</sup> and Luiz Antonio Carlos Bertollo<sup>2</sup>

### ABSTRACT

*Hoplerythrinus unitaeniatus* fish collected from the Negro river, Manaus region (AM) were characterized by a diploid number equal to 48 chromosomes. A female specimen, however, exhibited 72 chromosomes, with a karyotype consisting of 23 M-SM chromosome triplets and one ST-A chromosome triplet, thus representing a case of natural triploidy. In this triploid, the NORs were detected in triplicate and were found to be active in only one of the different types of chromosomes involved in nucleolar organization in this species.

### INTRODUCTION

A few cases of natural triploidy have been detected in fish, such as Mexican populations of *Poeciliopsis* (Schultz, 1967) and in *Poecilia formosa*, in which diploid and triploid forms were found (Prehn and Rasch, 1969). In both cases, gynogenesis was proposed as a mechanism of triploid maintenance.

Eventual triploids in diploid populations have been observed in some fish such as *Salmo gairdneri* (Cuellar and Uyeno, 1972), *Hesperoleucus symmetricus* (Gold and Avise, 1976), *Misgurnus anquicaudatus* (Ojima and Takai, 1979), *Cyprinus carpio* (Al-Sabti *et al.*, 1983), *Astyanax schubarti* (Morelli *et al.*, 1983), *Curimata modesta* (Venere and Galetti Jr., 1985), and *Eigenmannia sp* (Almeida Toledo *et al.*, 1985). In

---

<sup>1</sup> Departamento de Biologia Geral, Universidade Estadual de Londrina, 86051 Londrina, PR, Brasil.  
Send correspondence to L.G.-C.

<sup>2</sup> Departamento de Ciências Biológicas, Universidade Federal de São Carlos, 13560 São Carlos, SP, Brasil.

these cases, the fertilization of a non-reduced ovule ( $2n$ ) by a haploid spermatozoon ( $n$ ) is considered to be the most probable origin of the triploids observed.

In the present paper we describe a new occurrence of triploidy in *Hoplerythrinus unitaeniatus*, a fish of the family Erythrinidae which is currently being investigated in different cytogenetic studies (Bertollo and Moreira-Filho, 1981; Giuliano-Caetano, 1986; Giuliano-Caetano and Bertollo, 1988).

## MATERIAL AND METHODS

Twenty-two specimens of *Hoplerythrinus unitaeniatus* (15 females and 7 males) were collected from the Negro river near the city of Manaus (AM). The animals were injected with 0.05% colchicine at a proportion of 1 ml/100 g body weight and sacrificed 90 minutes later. Mitotic metaphases were obtained from kidney cells by the air-drying technique of Bertollo *et al.* (1978).

As in previous studies (Giuliano-Caetano and Bertollo, 1988), only two chromosome groups were considered, i.e. meta-submetacentrics (M-SM) and sub-telo-acrocentrics (ST-A) because of the difficulty in identifying several chromosome pairs in a more precise manner.

The nucleolar organizer regions (NORs) were detected by the technique of Howell and Black (1980).

## RESULTS

A diploid number equal to 48 characterizes the *Hoplerythrinus unitaeniatus* population from the region studied, which presents a polymorphic karyotype in terms of structural rearrangements (Giuliano-Caetano and Bertollo, 1988).

However, among the females examined, one exhibited  $2n = 3x = 72$  chromosomes (Figure 1), representing a case of natural triploidy. The karyotype of this female (Figure 2B), when compared with a normal diploid karyotype for the species (figure 2A), is characterized by 23 triplets of M-SM chromosomes and 1 triplet of ST-A chromosomes.

In some preparations, chromosome no. 16 (Figure 2B) showed a secondary constriction on the short arm, probably corresponding to a nucleolar organizer region, as visualized by silver nitrate staining (Figure 3).

## DISCUSSION

At least 4 distinct cytotypes have been observed among  $2n = 48$  animals from this population: cytotype A (48 M-SM chromosomes), cytotype B (46 M-SM

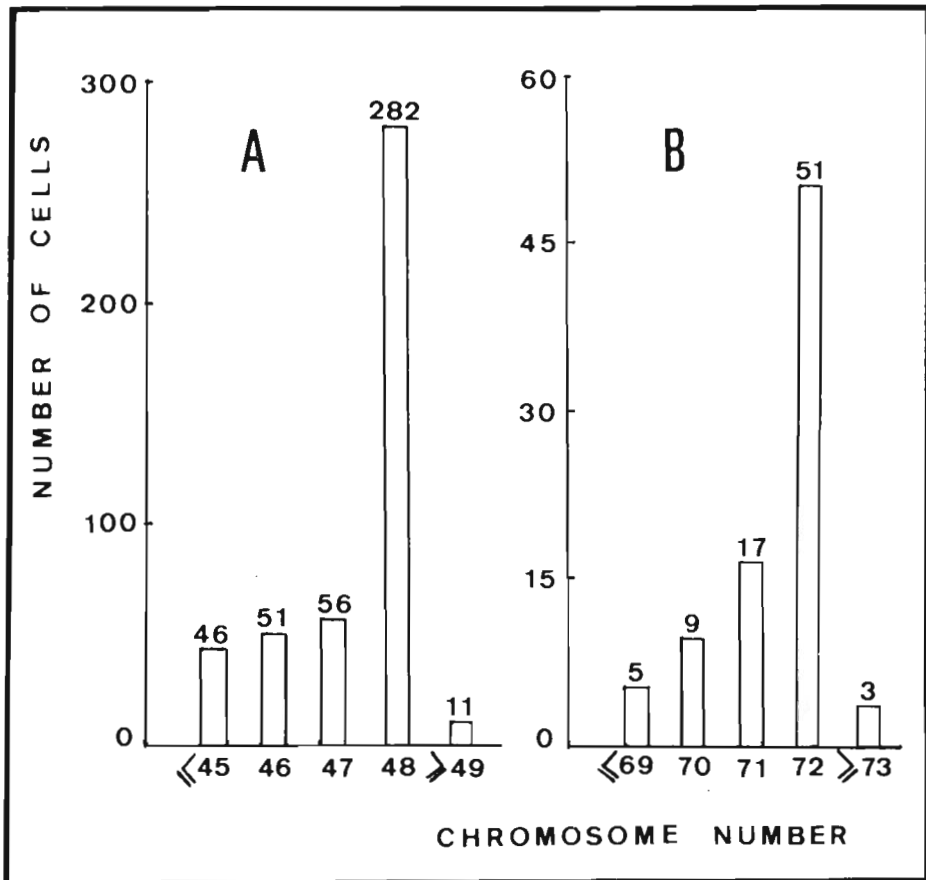


Figure 1 - Chromosome number frequency for *Hoplerhythrinus unitaeniatus* from the Negro river (AM). (A) Diploid specimen:  $2n = 2x = 48$  (data taken from Giuliano-Caetano and Bertollo, 1988). (B) Triploid specimen:  $2n = 3x = 72$ .

chromosomes and two relatively large ST-A of similar size), cytotype C (46 M-SM chromosomes and two ST-A chromosomes of different sizes, i.e., one of them large and the other small), and cytotype D (47 M-SM chromosomes and one relatively large ST-A chromosome) (Giuliano-Caetano and Bertollo, 1988).

The karyotype of the triploid specimen described here showed good agreement between the M-SM chromosome triplets and the chromosome pairs of a diploid specimen (cytotype B) taken as reference (Figure 2). In turn, the ST-A triplet included distinct chromosomes, one of which was clearly smaller, whereas the other two were generally more similar in size to one another. Thus, it is evident that at least one of the animals involved in the fertilization that gave origin to this triploid should

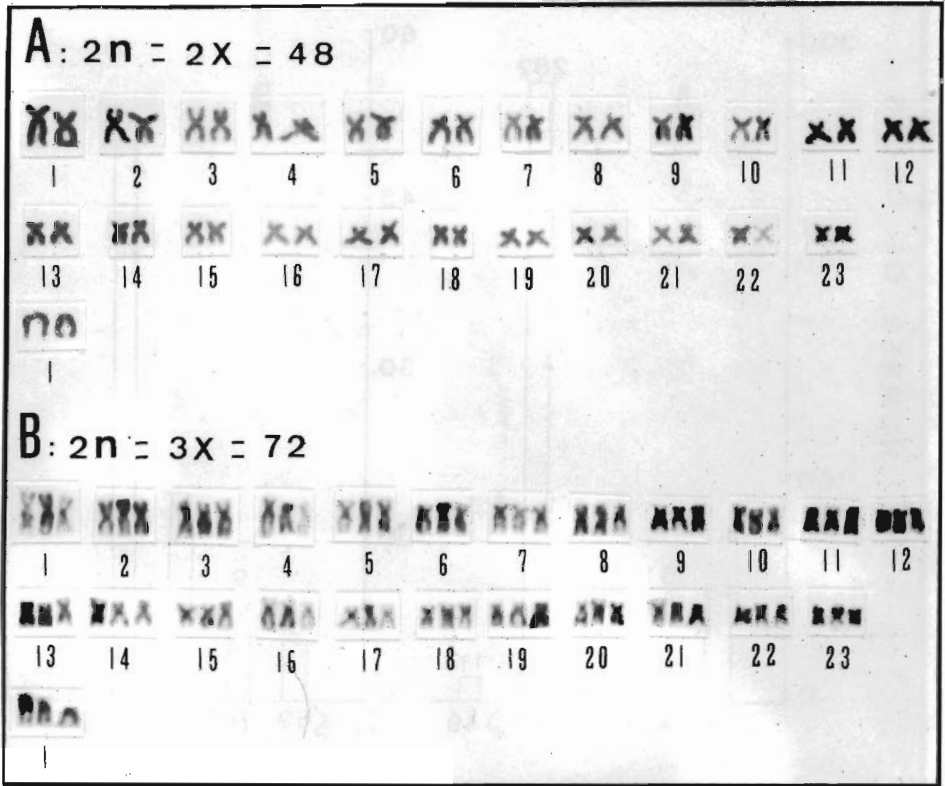


Figure 2 - Karyotypes of *Hoplerythrinus unitaeniatus* from the Negro river (AM). (A) Diploid specimen (cytotype B, with chromosomes paired according to data from Giuliano-Caetano and Bertollo, 1988). (B) Triploid specimen. In each karyotype, the chromosomes in the two upper rows belong to the M-SM group, and those in the third row, to the ST-A group.

have borne the small-sized ST-A chromosome. Different karyotypes may also be related to this occurrence, producing a non-reduced gamete (possibly the ovule) and a normal haploid gamete.

Since temperature is an important factor in the experimental derivation of polyploids (Purdom, 1972; Purdom and Lincoln, 1973; Valenti, 1975), the number of triploid cases currently being detected among fish may be correlated with the biological characteristics of this animal group, being directly affected by the environmental temperature and by the conditions of fecundation, which is external in most species.

In *Eigenmannia sp.*, which has a single NOR-bearing homologue pair, these regions remained active in the three corresponding chromosomes of a triploid specimen detected by Almeida Toledo *et al.* (1985), indicating the absence of

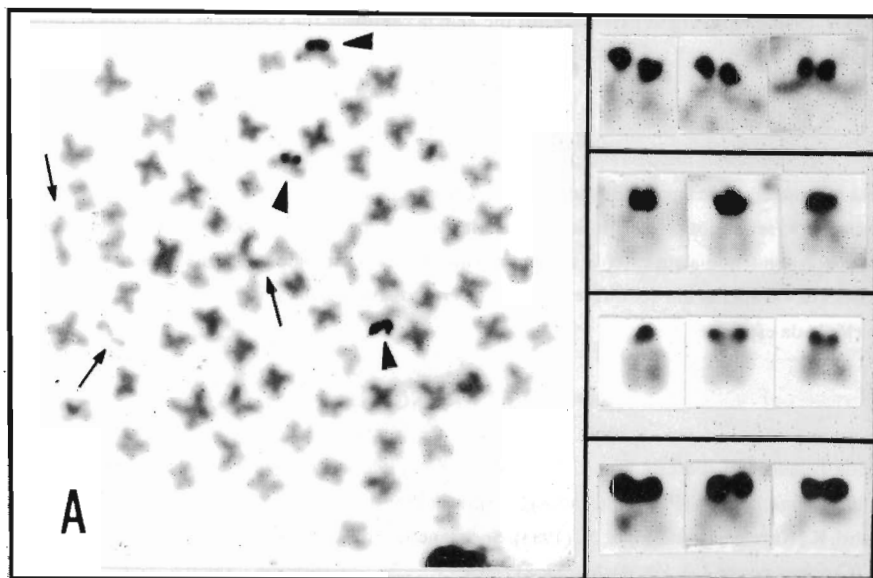


Figure 3 - Nucleolar organizer regions of the triploid *Hoplerythrinus unitaeniatus* specimen from the Negro river (AM). (A) Metaphase showing the three NOR-bearing chromosomes (arrowheads) and the three ST-A chromosomes (arrows). On the right, details of the chromosome triplet with the NORs, from four distinct metaphases.

ribosome cistron inactivation under triploid conditions. In the case of *Curimata modesta*, the presence of cells with three and two NORs in a triploid animal suggests the evidence of a process of gene regulation in these regions (Venere and Galetti Jr., 1985).

*H. unitaeniatus* has multiple NORs that may be located in a terminal or interstitial position depending on the chromosome involved with nucleolar organization (Giuliano-Caetano, 1986). In the specimen described here, three chromosomes had terminal NORs on the short arm, probably triplet no. 16 which has a secondary constriction in this region (Figures 2B and 3). The NORs of the remaining nucleolar chromosomes did not show any activity. On this basis, the activity of a specific type of NORs among the set of NORs existing in the species was demonstrated, at least in the metaphases analyzed.

#### ACKNOWLEDGMENTS

Research supported by CNPq (PIG), Federal University of São Carlos and State University of Londrina. The authors are also grateful to the National Research Institute of the Amazon Region (INPA),

and to Dr. Eliana Feldberg in particular, for the help in capturing the specimens. Publication supported by FAPESP.

## RESUMO

*Hoplerythrinus unitaeniatus*, coletados no rio Negro, região de Manaus (AM), caracterizam-se por um número diplóide igual a 48 cromossomos. Um exemplar fêmea, entretanto, apresentou 72 cromossomos, sendo o seu cariótipo constituído por 23 trincas de cromossomos M-SM e 1 trinca de cromossomos ST-A, correspondendo a um caso de triploidia natural. Nesse triploide, as NORs mostraram-se ativas e em triplicata, em apenas um dos diferentes tipos de cromossomos que estão envolvidos com a organização do nucléolo da espécie.

## REFERENCES

- Almeida Toledo, L.F., Foresti, F. and Toledo Filho, S.A. (1985). Spontaneous triploidy and NOR activity in *Eigenmannia* sp (Pisces, Sternopygidae) from the Amazon basin. *Genetica* 66: 85-88.
- Al-Sabti, K., Kurelec, B. and Fijan, N. (1983). Spontaneous triploidy and tetraploidy in the common carp (*Cyprinus carpio* L.). *Vet. Archiv.* 53: 217-223.
- Bertollo, L.A.C. and Moreira-Filho, O. (1981). Estudos citogenéticos em populações do gênero *Hoplerythrinus* (Pisces, Erythrinidae). *Ciênc. Cult.* 33: 695 (abstract).
- Bertollo, L.A.C., Takahashi, C.S. and Moreira-Filho, O. (1978). Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Rev. Brasil. Genet.* 1: 103-120.
- Cuellar, O. and Uyeno, T. (1972). Triploidy in rainbow trout. *Cytogenetics* 11: 508-515.
- Giuliano-Caetano, L. (1986). Estudo citogenético em *Hoplerythrinus unitaeniatus* (Pisces, Erythrinidae) de diferentes bacias hidrográficas brasileiras. Masters Thesis. Universidade Federal de São Carlos, São Carlos.
- Giuliano-Caetano, L. and Bertollo, L.A.C. (1988). Karyotype variability in *Hoplerythrinus unitaeniatus* (Characiformes, Erythrinidae). I. Chromosome polymorphism in the rio Negro population (Manaus, State of Amazonas). *Rev. Brasil. Genet.* 11: 299-306.
- Gold, J.R. and Avise, J.C. (1976). Spontaneous triploidy in the California roach *Hesperoleucus symmetricus* (Pisces, Cyprinidae). *Cytogenet. Cell Genet.* 17: 144-149.
- Howell, W.N. 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.
- Morelli, S., Bertollo, L.A.C. and Moreira-Filho, O. (1983). Cytogenetic considerations on the genus *Asytanax* (Pisces, Characidae). II. Occurrence of natural triploidy. *Caryologia* 36: 245-250.
- Ojima, Y. and Takai, A. (1979). The occurrence of spontaneous polyploid in the japanese common loach, *Misgurnus anguicaudatus*. *Proc. Japan Acad.* 55: 487-491.
- Prehn, L.M. and Rasch, E.M. (1969). Cytogenetic studies of *Poecilia* (Pisces). I. Chromosome numbers of naturally occurring poeciliid species and their hybrids from eastern Mexico. *Can. J. Genet. Cytol.* 11: 880-895.
- Purdom, C.E. (1972). Induced polyploidy in plaice (*Pleuronectes platessa*) and its hybrid with the flounder (*Platichthys flesus*). *Heredity* 29: 11-24.

- Purdom, C.E. and Lincoln, R.F. (1973). Chromosome manipulation in fish. In: *Genetics and Mutagenesis of Fish* (Schroder, J.H., ed.). Springer-Verlag, Berlin, pp. 83-89.
- Schutz, R.J. (1967). Gymnogenesis and triploidy in the viviparous fish *Poeciliopsis*. *Science* 157: 1564-1567.
- Valenti, R.J. (1975). Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatment. *J. Fish Biol.* 7: 519-528.
- Vêneré, P.C. and Galetti Jr., P.M. (1985). Natural triploidy and chromosome B in the fish *Curimata modesta* (Curimatidae, Characiformes). *Rev. Brasil. Genet.* VIII: 681-687.

(Received July 27, 1989)