



Natural triploidy in *Leporinus* cf. *elongatus* bearing sex chromosomes

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Abstract

Although several cases of natural triploidy in fish have already been described, spontaneous polyploidy in species with differentiated sex chromosomes are rare. We report the occurrence of a triploid fish ($3n = 81$) *Leporinus* cf. *elongatus*, a species characterized by a highly differentiated ZZ/ZW sex chromosome system, from the São Francisco river. The occurrence of a ZZZ triploid adult indicates the viability of this chromosome constitution in this fish.

Key words: polyploidy, triploid fish, ZW chromosomes, sex chromosomes, Anostomidae.

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The family Anostomidae is composed of 12 genera, constituting a group relatively rich in species, distributed from South to Central America (Garavello, 1979). Eight species from this group, all from the genus *Leporinus*, exhibit a ZZ/ZW sex chromosome system with marked structural modifications between the sex chromosomes (Galetti *et al.*, 1995; Venere *et al.*, 2004; Molina and Galetti, 2006). The ZW chromosomes of these species point a monophyletic origin, apparently differentiated through processes of heterochromatinization (Galetti and Foresti, 1986; 1987). *Leporinus* is one of the more complex groups among Characiformes and the largest in number of species, with 87 already described. Several attempts to subdivide it into additional genera and subgenera have failed (Garavello and Britski, 2003).

A diploid number of $2n = 54$ is common among *Leporinus* species (Galetti *et al.*, 1995) and no chromosome number variation has been reported in this genus thus far, despite of several reports of natural triploidy in different neotropical fish families (Characidae, Morelli *et al.*, 1983; Fauaz *et al.*, 1994; Centofante *et al.* 2001; Sternopygidae, Almeida-Toledo *et al.*, 1985; Curimatidae, Venere and Galetti, 1985; Erythrinidae, Giuliano-Caetano and Bertollo, 1990; Thricomycteridae, Borin *et al.*, 2002).

This study reports a case of spontaneous natural triploidy in *Leporinus* cf. *elongatus* with a highly differentiated ZZ/ZW sex chromosome system.

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A total of 28 specimens of *Leporinus* cf. *elongatus* (12 males and 16 females) collected in the São Francisco river (Três Marias municipality; Brazil) were cytogenetically analyzed through the air-drying technique (Bertollo *et al.*, 1978; Gold *et al.*, 1990). The chromosome preparations were submitted to Ag-NOR (Howell and Black, 1980) and mithramycin staining (Schweizer, 1980).

Our results confirmed the previously reported $2n = 54$ with a highly differentiated ZZ/ZW sex chromosome system, characterized by a large subtelocentric W chromosome and a submetacentric Z (Molina *et al.*, 1998).

A young triploid male with $2n = 3x = 81$ and presenting three copies of the Z chromosome was detected (Figure 1a, b). A previously reported conspicuous mithramycin positive heterochromatic block on the long arm of the Z chromosomes (Molina *et al.*, 1998) was evident in the three Z chromosomes of the triploid complement (Figure 1c). No morphological differences were observed between the triploid and the diploid specimens.

The nucleolus organizer regions (NORs) were located near the telomere of the long arm of a large submetacentric chromosome in the diploid individuals (Figure 1d). Although good quality metaphases were not obtained for Ag-staining in the triploid specimen, while the interphase nuclei of diploid individuals has showed a maximum of two Ag-stained nucleoli in the triploid three nucleoli were detected (Figure 1e), indicating that these three constitutive NOR sites can be active in the triploid. Similar results were also observed in *Trichomycterus davisi* (Borin *et al.*, 2002).

Polyploidy is believed to be an important mechanism in the chromosome evolution in some fish groups, such as Catostomidae and Salmonidae (reviewed in Comber and Smith, 2004) or in the neotropical Siluriformes, as *Corydoras*, *Aspidoras* and *Brochis* (Oliveira *et al.*, 1993). Therefore, the polyploidy is particularly propitious to the evolutionary failure in species with differentiated sex chromosomes, likely due to a gene imbalance (Müller, 1925; Svartman *et al.*, 2005). On the other hand, random natural triploidy appears more frequent in fish and it is mostly correlated to exposition to drastic temperature changes or other environmental conditions (Venere and Galetti, 1985), once the fecundation most of the time is external. However, natural triploidy in fish species with differentiated sex chromosomes is very rare.

In a set of 27 individuals of *Characidium gomesi*, which presents a ZZ/ZW system, Centofante *et al.* (2001)

reported the occurrence of a triploid female. This triploid exhibited one W and two Z chromosomes, suggesting that it resulted from the fertilization of a normal (haploid) ovule by a non-reduced spermatozoid (2n). The presence of three Z chromosomes in the triploid male herein studied prevented us from determining if the triploidy was originated by maternal or paternal non-segregation.

The occurrence of triploids in cytogenetic analyses with samples relatively small as in *L. cf. elongatus* from the São Francisco river suggests that polyploidy events should happen in a larger frequency than could be imagined. However, one of the limitations to the evolution through the polyploidy in vertebrates has been attributed to the presence of differentiated sex chromosomes (Svartman *et al.*, 2005).

The presence of sex chromosomes does not seem to impair the triploid development in *L. cf. elongatus* and our

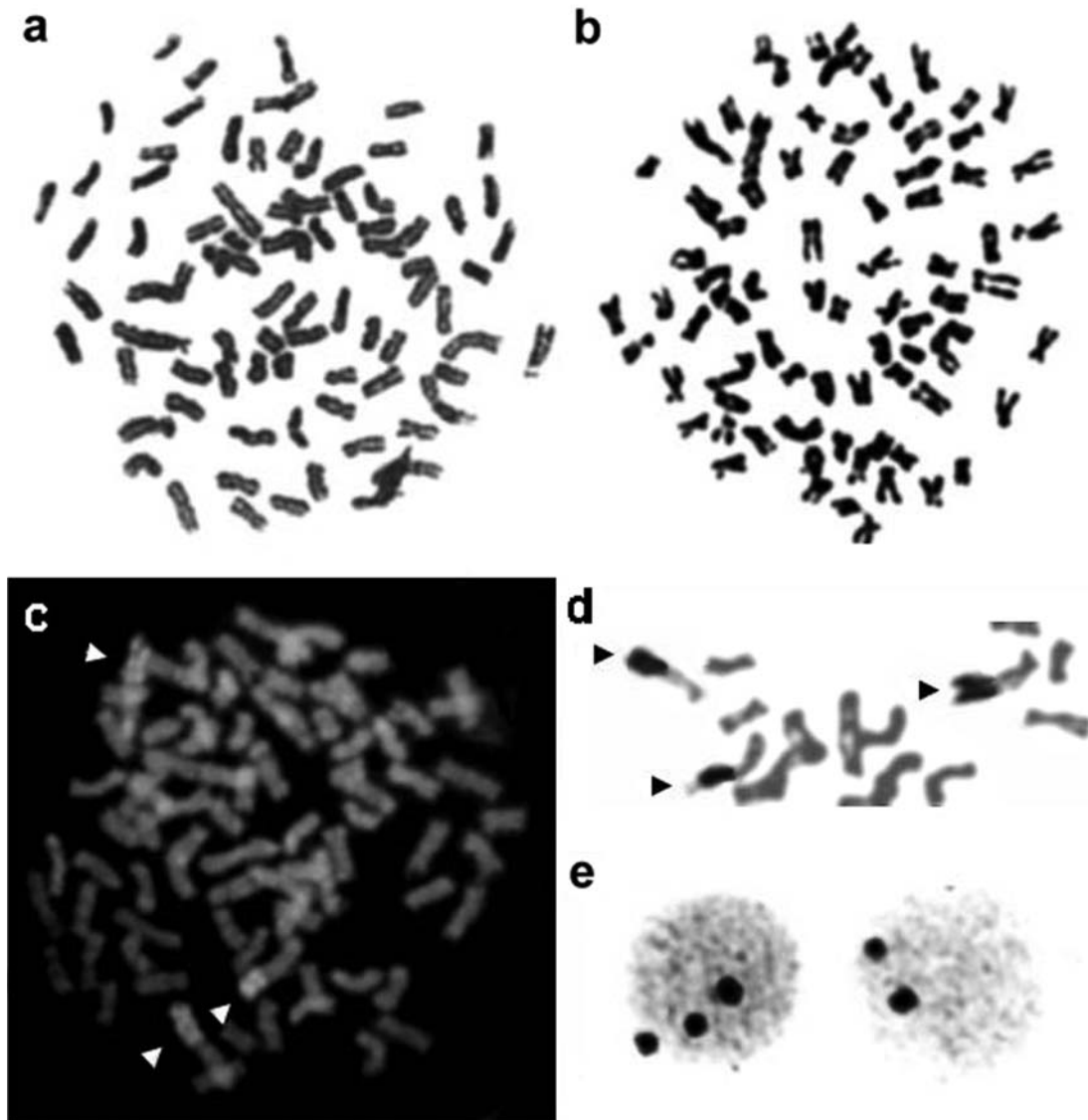


Figure 1 - (a-c) Metaphases of a triploid male *L. cf. elongatus*. (a-b) Giemsa staining. (c) Mithramycin staining. Arrows indicate the Z chromosomes. (d) Ag-stained partial diploid metaphase with two Ag-NOR bearing chromosomes. (e) Ag-stained nucleoli in the triploid specimen.

findings may have direct implications on the genetic manipulation of this fish and of other species bearing differentiated sex chromosomes.

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