

Differentiation and evolutionary relationships in *Erythrinus erythrinus* (Characiformes, Erythrinidae): occurrence and distribution of B chromosomes

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ABSTRACT. *Erythrinus erythrinus*, a Neotropical fish species of the Erythrinidae family, has a wide distribution in South America. Previous cytogenetic analysis showed that this species presents extensive karyotype diversity, with 4 karyomorphs (A-D) described herein. This study investigated the karyotypic structure of 2 new populations of *E. erythrinus* from the Brazilian Pantanal region, in order to improve the knowledge of the chromosomal diversity in this species. Both populations showed typical characteristics of karyomorph A, with $2n = 54$ chromosomes ($6m + 2st + 46a$), without differentiation between males and females. In addition, identical supernumerary B chromosomes, appearing as double-minute chromosomes, were also found in both populations. These findings suggest the presence of mitotic instability in view of their high intra- and inter-individual numerical variation. The

presence of these chromosomes is likely a basal characteristic for this group, since the same kind of Bs also occurs in some other populations and karyomorphs of *E. erythrinus*. As such, they are important markers of biodiversity found in this nominal species, which probably corresponds to a species complex.

Key words: Fish; Species complex; Chromosomal diversity; Supernumerary chromosomes

INTRODUCTION

Erythrinidae is a small Neotropical fish family, with only 3 recognized genera, i.e., *Hoplias*, *Hopleryrinus*, and *Erythrinus*. The genus *Erythrinus* includes only 2 nominal species: *Erythrinus erythrinus*, which has a wide distribution in South America, and *Erythrinus kessleri* with a restricted distribution (Oyakawa, 2003). Cytogenetic studies conducted on *E. erythrinus* indicate that this species is probably a species complex, with 4 karyomorphs (A-D) differing both in chromosomal number and morphology, as well as in the occurrence of heteromorphic sex chromosomes (reviewed in Cioffi et al., 2012). Karyomorph A presents $2n = 54$ chromosomes [6 metacentrics (m) + 2 subtelocentrics (st) + 46 acrocentrics (a)] in males and females, without submetacentric (sm) chromosomes and heteromorphic sex chromosomes. The other 3 karyomorphs (B-D) share a multiple $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system, but have different karyotypic formulas. Karyomorph B has $2n = 54$ chromosomes in females and $2n = 53$ chromosomes in males ($\text{♀ } 6m + 2st + 46a$; $\text{♂ } 7m + 2st + 44a$); karyomorph C has $2n = 52$ chromosomes in females and $2n = 51$ chromosomes in males ($\text{♀ } 6m + 2sm + 6st + 38a$; $\text{♂ } 7m + 2sm + 6st + 36a$); and karyomorph D has $2n = 52$ chromosomes in females and $2n = 51$ chromosomes in males ($\text{♀ } 4m + 2sm + 2st + 44a$; $\text{♂ } 5m + 2sm + 2st + 42a$).

Small supernumerary B chromosomes, morphologically similar to “double-minute chromosomes”, have already been found in some populations of *E. erythrinus* (Bertollo et al., 2004). Since their discovery in the last century (Wilson, 1907), supernumerary chromosomes have been the subject of many investigations in respect to their origin and biological significance (Camacho et al., 2000). Although occurring in several biological groups (Jones and Rees, 1982), they are dispensable elements usually not following the Mendelian inheritance pattern, with an irregular segregation during meiosis (Jones and Rees, 1982; Buekeboom, 1994; Camacho et al., 2000). B chromosomes commonly have smaller sizes and distinct morphologies in relation to the standard chromosomes (A) of the karyotype, and their evolutionary process is usually associated with the occurrence of heterochromatinization and accumulation of repetitive DNAs (Camacho et al., 2000).

In this study, the karyotype structure of new populations of *E. erythrinus*, which have not been yet investigated, from 2 Brazilian regions were analyzed. The main objective of this study is to improve the knowledge of chromosomal diversity and geographic distribution of the karyomorphs in this fish species. The results indicated broader participation of B chromosomes in the karyotype diversification of *E. erythrinus* and reinforced that this nominal species represents, in fact, an assemblage of species with unresolved taxonomy.

MATERIAL AND METHODS

Specimens

Forty specimens of *E. erythrinus* from 2 Brazilian sites were analyzed. In all, 16 fe-

males and 14 males were from the Barão de Melgaço region (16°10'16.67"S, 55°57'26.28"W) and 7 females and 3 males were from the Poconé region (16°15'48.32"S; 56°37'48.64"W; Figure 1). Both sites are situated in the Pantanal of the Mato Grosso State, which comprises a mostly flooded extensive area of 250,000 km² in the Midwest of Brazil.



Figure 1. Collection sites of two Brazilian *Erythrinus erythrinus* samples analyzed in this study. 1 = Poconé region; 2 = Barão de Melgaço region.

Mitotic chromosome preparations

The specimens were first treated with a 0.005% colchicine solution (1 mL solution/100 g body weight) for a period of 40-50 min. Next, they were anesthetized, and their anterior kidney was removed; the tissues were dissociated to obtain a homogeneous suspension of cells. The metaphase chromosomes were obtained according to the conventional air-drying method (Bertollo et al., 1978). In brief, cells were treated with a hypotonic KCl solution (0.075 M) for 20 min at 36°C and fixed 3 times in methyl alcohol (3 parts): glacial acetic acid (1 part). The metaphase plates were analyzed by light microscopy (Olympus BX50), and images were captured using the CoolSNAP-Pro software (Media Cybernetics). At least 30 metaphases from each specimen were analyzed. The chromosomes were classified as m, sm, st, and a, according to the arm ratio (Levan et al., 1964).

RESULTS

Both populations showed the same karyotype characteristics, i.e., $2n = 54$ chromosomes (6m + 2st + 46a) without differentiation between males and females (Figure 2). Additionally, they also showed supernumerary B chromosomes, which usually appeared like double-minute chromosomes and stained less than the standard chromosomes. There were 0-6 B chromosomes per cell, considering the sample of 521 metaphases analyzed. Cells not carrying Bs were the most frequent (38.9%), followed by cells carrying 1B (20.9%) and 2Bs (20.5%). Cells carrying 3 to 6 Bs had lower frequency, approximately, 19.7% of the sample. Accordingly, cells with 1 or 2 Bs corresponded to 41.4% of the total sample analyzed, unlike cells carrying a larger number of Bs (Figures 3 and 4).

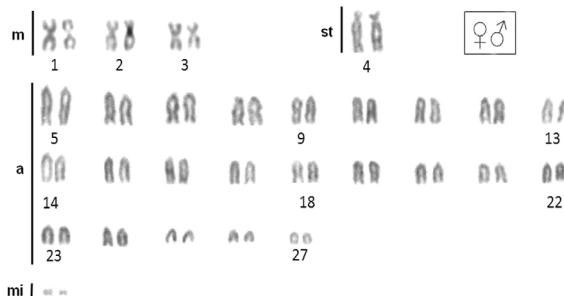


Figure 2. Male and female karyotypes for both *Erythrinus erythrinus* populations analyzed, showing $2n = 54$ chromosomes ($6m + 2st + 46a$) and two supernumerary B chromosomes. m = metacentric; st = subtelocentric; a = acrocentric; mi = B chromosomes. Bar = 5 μ m.

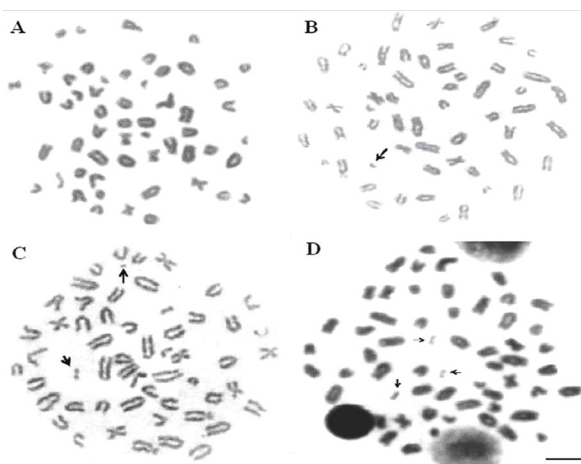


Figure 3. Metaphase chromosomes representative of both *Erythrinus erythrinus* populations investigated, showing no B chromosomes (A), one B chromosome (B), two B chromosomes (C), and three B chromosomes (D) indicated by arrows. Bar = 5 μ m.

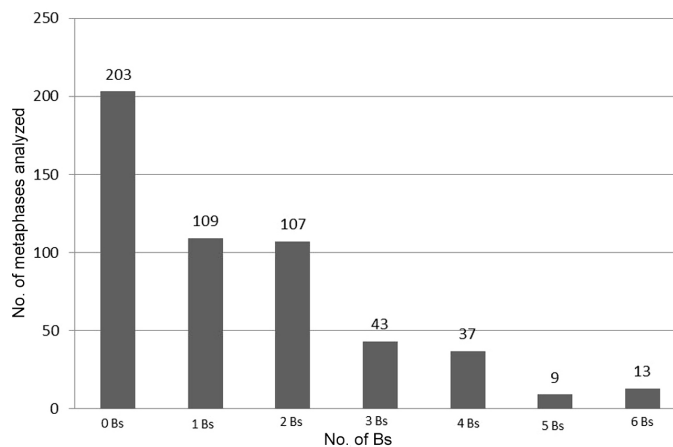


Figure 4. Frequency of B chromosomes found in the two populations of *Erythrinus erythrinus*. Note the higher frequency of 1-2 Bs/cell, the intermediate frequency of 3-4 Bs/cell, and the lower frequency of 5-6 Bs/cell.

DISCUSSION

The 2 new populations of *E. erythrinus* showed the same karyotype, highlighting the typical chromosomal features of karyomorph A (Bertollo et al., 2004). These data showed that this karyomorph has a wider distribution than the other karyomorphs. In fact, while karyomorph A was already found in the Southeast and Midwest Brazilian regions and Northern Argentina, the karyomorphs B-D were only more punctually located (Bertollo et al., 2004 and the present study).

Micro-B chromosomes were also found in both populations. However, these chromosomes are not exclusive to the investigated populations, since they are also found in some other populations of karyomorphs A and B. Previous studies have indicated that karyomorph A might represent the basal condition in *E. erythrinus*, from which the other karyomorphs would have diversified (Bertollo et al., 2004). Among these, karyomorph B shows a greater proximity to karyomorph A, retaining the same number of chromosomes in females ($2n = 54$), but reducing it in males ($2n = 53$) due the differentiation of a multiple $X_1X_1X_2X_2/X_1X_2Y$ sex chromosome system (Bertollo et al., 2004). The occurrence of the same type of Bs among different karyomorphs and populations from different geographic regions is indicative of the ancestry of these chromosomes. Therefore, they might represent a basal characteristic in *E. erythrinus* and are now found among some populations of different karyomorphs, with or without differentiated sex chromosomes (Figure 5). In fact, one of the main properties of B chromosomes is the non-universality, i.e., their independent occurrence among some individuals of some populations of a species, following a particular evolutionary pathway (Camacho et al., 2000). In this context, populations of different karyomorphs, in addition to those already investigated, are expected to present these additional chromosomes, thereby raising questions regarding their possible roles.

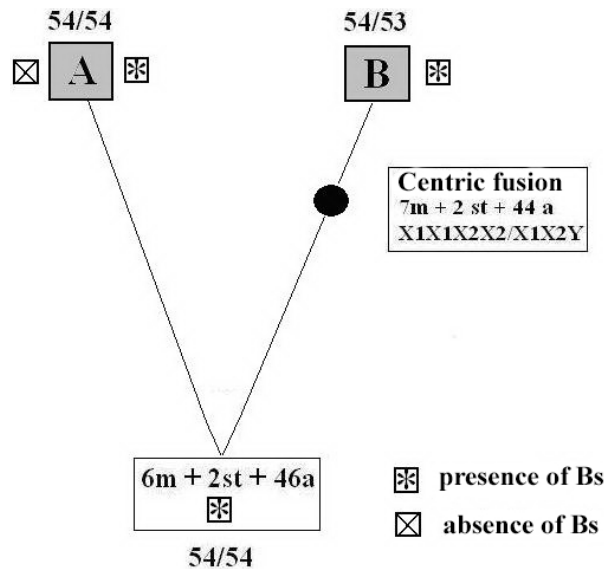


Figure 5. Evolutionary differentiation of *Erythrinus erythrinus* populations from karyomorphs A ($2n = 54$ chromosomes, without differentiated sex chromosomes) and B ($2n = 54$ chromosomes in females, $X_1X_1X_2X_2$ and $2n = 53$ chromosomes in males, X_1X_2Y), based on their chromosomal characteristics. Note the occurrence of micro-B chromosomes shared by some populations of the karyomorphs A and B.

In general, the absence of phenotypic traits correlated with the presence of Bs suggests that they are genomic parasites without benefits for their carriers (Camacho et al., 2000). However, some specific situations indicate probable roles performed by these chromosomes, as in the cichlid species from Lake Victoria (Africa), where a female-specific B chromosome carries several genes encoding proteins, highlighting its possible functional effect on female sex determination (Yoshida et al., 2011). Likewise, in *Chilomycterus spinosus* (Tetraodontiformes), a B chromosome is restricted to males and behaves as a univalent during meiosis, also suggesting its correlation with sex differentiation in this species (Noieto et al., 2012). Similarly, in *Astyanax scabripinnis* (Characiformes), B chromosomes have a major occurrence among females, besides their altitudinal variation in frequency (Néo et al., 2000a,b), suggesting possible adaptive effects.

B chromosomes are known to occur in several fish species. According to the revision study of Carvalho et al. (2008), supernumerary chromosomes were recorded for 61 fish species, predominantly belonging to distinct families of the orders Characiformes and Siluriformes. These chromosomes have different morphologies and sizes, ranging from very small like in *Prochilodus* (Pauls and Bertollo, 1983) and *Moenkhausia* (Foresti et al., 1989), medium sized as in *Rhamdia* (Fenocchio and Bertollo, 1990), to the largest ones of the complement as in *Astyanax* (Salvador and Moreira-Filho, 1992; Maistro et al., 1992). In some species, there is structural and functional evidence that B chromosomes have an intraspecific origin, for example, the large isochromosome found in *A. scabripinnis* (Vicente et al., 1996; Mestriner et al., 2000; Vicari et al., 2011). Bs also seems to have an intraspecific origin in the species *Prochilodus lineatus* considering the chromosomal markers derived from repetitive DNA (de Jesus et al., 2003; Artoni et al., 2006). At present, information concerning the intra- or inter-specific origin of supernumerary chromosomes found in *E. erythrinus* is lacking. However, in view of their characteristics, it is likely that they could have arisen from chromosomal rearrangements occurred during the evolutionary history of this species.

Mitotic instability is another noteworthy attribute of most B chromosomes (Jones and Rees, 1982). However, there are also several records indicating stable mitotic behavior for these chromosomes, as in *A. scabripinnis* where a macro-B chromosome is permanently found in the cells of the carriers (Moreira-Filho et al., 2004). In addition, in *P. lineatus*, unstable B chromosomes have become mitotically stable over time. Populations of this species usually have from 0 to 7 small B chromosomes (Pauls and Bertollo, 1983; Oliveira et al., 1997; Cavallaro et al., 2000; Artoni et al., 2006), which may, however, reach up to 9 in experimental crosses (Voltolin et al., 2011). In a natural population of *P. lineatus*, the frequency of B chromosomes doubled over few years. Concomitantly, the instability of these chromosomes had a marked decrease, suggesting that the mitotic stabilization might be the reason why unstable B chromosomes may become neutralized (Cavallaro et al., 2000). Indeed, this neutralization was also supported by the value of their transmission rate ($KB \approx 0.5$), indicating a Mendelian mode of inheritance (Oliveira et al., 1997). The numerical variation of Bs in *E. erythrinus* shows that they are also mitotically unstable in this species. In fact, variations occurred between not only the different specimens analyzed, but also the different metaphases of the same specimen.

The particular chromosomal characteristics of the karyomorphs of *E. erythrinus* and their geographic distributions reinforce that this nominal species probably corresponds to a species complex. Indeed, its biodiversity is evidenced by the distinct chromosomal rearrangements that are a characteristic of each karyomorph (Bertollo et al., 2004; Cioffi et al., 2010),

including the origin of a multiple sex chromosome system and the supernumerary chromosomes presently found in some populations.

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