Research Article

Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5s rRNA genes

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Abstract

Cytogenetic studies were done on three Pomacentridae species of the genus *Chromis*. The karyotype of *C. multilineata* consisted of 48 acrocentric chromosomes (FN = 48), *C. insolata* had 2n = 46-47 (3-4M+6SM+36-38A; FN = 56) and *C. flavicauda* had 2n = 39 (9M+6SM+24A; FN = 54). Robertsonian polymorphisms were detected in *C. insolata* and *C. flavicauda*. All three species had small heterochromatic blocks restricted to centromeric regions. Nucleolar organizer regions (NORs) were detected in the telomeric position of a medium acrocentric chromosome pair in *C. multilineata* and in non-homologous chromosomes in both *C. flavicauda* and *C. insolata*. FISH with a telomeric probe detected no internal telomeric sequences in *C. flavicauda* and *C. insolata*. 5S rRNA genes were observed in a pericentromeric region of two large metacentric chromosome pairs in *C. flavicauda* and two large acrocentric pairs in *C. insolata*. The karyotype structure and the number and location of the 5S rDNA loci in these two species indicated that the 5S rRNA-bearing acrocentric chromosomes were directly involved in the origin of the polymorphisms observed. These data reinforce the idea that Robertsonian rearrangements have been involved in molding the karyotype in the subfamily Chrominae.

Key words: *Chromis*, FISH, polymorphism, Robertsonian rearrangements, 5S rDNA, telomeric sequences.

Introduction

Genetic differentiation in populations of reef fishes has been shown by allozyme (Knowlton *et al.*, 1993) and mitochondrial DNA (Knowlton *et al.*, 1993; Shulman and Bermingham, 1995). In contrast, several groups of these fish have quite a conservative chromosomal structure, even when large geographical distances are considered (for a review, see Brum and Galetti, 1997). Robertsonian chromosomal rearrangements, such as centric fusion and fission, have been identified as important mechanisms in the karyotype differentiation of distinct fish groups (Hartley and Horne, 1984; Amores *et al.*, 1990).

In reef fish belonging to the Pomacentridae, centric fusion has been described either as a polymorphic condition, as observed in the genus *Dascyllus* (Ojima and Kashiwagi, 1981), or established in derived karyotypes, as detected in two species of *Chrysiptera* (Takai and Ojima,

1995). Robertsonian chromosomal rearrangements have also been implicated in the origin of novel, multiple sex chromosome systems in fish (Brum *et al.*, 1992) and in some cases, may contribute to the occurrence of genetically isolated populations.

In this paper, we describe the karyotype, heterochromatin pattern and nucleolar organizer regions in three Pomacentridae, *Chromis multilineata*, *C. insolata* and *C. flavicauda*. In addition, telomeric- and 5S rDNA probes were used in fluorescence *in situ* hybridization (FISH), to identify the chromosomes involved in the Robertsonian rearrangements observed in *C. insolata* and *C. flavicauda*.

Materials and Methods

Cytogenetic analyses were done on three Pomacentridae species from two regions in Brazil. *Chromis multilineata* (1 juvenile and 3 females) were collected on the coast of the State of Bahia and *C. insolata* (4 juveniles and 4 females) and *C. flavicauda* (2 males) were obtained from the State of Espírito Santo. Chromosomal preparations were obtained as described by Bertollo *et al.* (1978).

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mitosis was stimulated by the intraperitoneal injection of yeast glucose solution (Lee and Elder, 1980). Ag-NORs and C-bands were detected as described by Howell and Black (1980) and Sumner (1972), respectively.

Fluorescence *in situ* hybridization (FISH) using chromosomes of *C. insolata* and *C. flavicauda* was done as described by Martins and Galetti (1999). Telomeric sites were detected using the telomeric probe $(TTAGGG)_n$ of *Xenopus laevis*, in either high or low formamide stringency conditions (70% or 30%, respectively). The chromosomal localization of 5S rDNA was determined using a pBSIIKS recombinant plasmid containing the 5S rRNA gene plus its non-transcribed spacer (NTS) obtained from the fish *Leporinus elongatus* (Martins and Galetti, 1999). Both probes were labeled with biotin-11-dUTP by nick translation according to the manufacturer's instructions (Bionick^{TM} Labeling System, Gibco). The 5S rDNA loci and telomeric sites were visualized using biotinylated anti-avidin antibody and an avidin-fluorescein isothiocyanate (FITC) conjugate. Metaphases were examined with an Olympus BX50 epifluorescence microscope and photographed with 400 ASA Kodak Ektachrome film.

Results

Chromis multilineata had an invariant karyotype of 48 acrocentric chromosomes, with a fundamental number (FN) of 48 (Figure 1a). In *C. insolata*, two cytotypes were observed, each differing in the number of metacentric chromosomes. Four individuals showed 2n = 46 (4M+6SM+36A; FN = 56) and four had 2n = 47 (3M+6SM+38A; FN = 56) (Figure 1b). The two individuals of *C. flavicauda* showed a karyotype with 2n = 39 and FN = 54 (9M+6SM+24A) (Figure 1c). Both *C. insolata* and *C. flavicauda* had large metacentric chromosomes that were almost double the size of the other chromosomes of the complement. A peculiar radial configuration of the chromosomes was frequently observed in the metaphase figures of all the species analyzed (Figure 1d, e).

Ag-NORs were detected on the short arm of a medium-size acrocentric chromosomal pair in *Chromis multilineata* (Figure 1a) and in the telomeric portion of the short arm of two non-homologous acrocentric chromosomes of *C. insolata* (Figure 1b). In the latter species, an additional NOR was occasionally observed in the proximal region on the long arm of a large acrocentric chromosome. In *C. flavicauda*, NORs were detected at the telomeric position of distinct chromosomal pairs. One site was located on the short arm of a small acrocentric chromosome, while the other was located on the long arm of a small submetacentric (Figure 1c).

All three species showed a common C-banding pattern characterized by small centromeric blocks of heterochromatin on most chromosomes (Figure 2a, b, c).

At low stringency, the telomeric probe gave conspicuous signals on the terminal portion of practically all *C.*
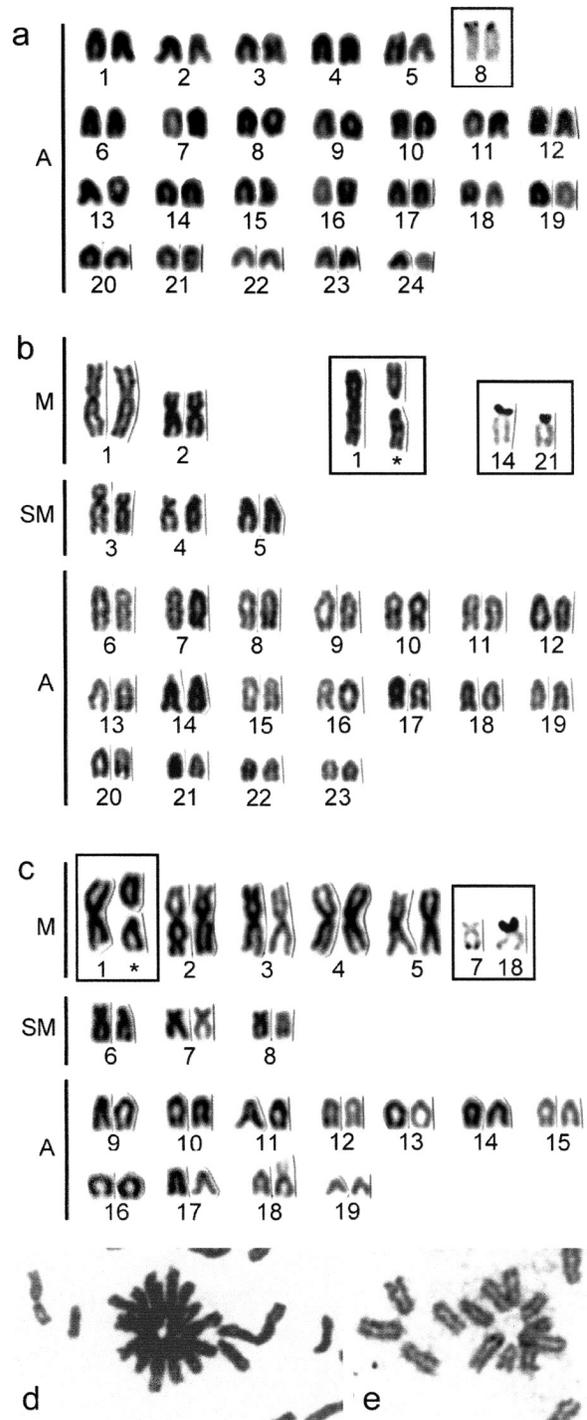
Figure 1 - Giemsa stained karyotypes of *Chromis multilineata* (a), *C. insolata* (b) and *C. flavicauda* (c) with chromosomes related to centric fusion indicated in the larger boxes. The Ag-NOR bearing chromosomes are in the smaller boxes. Partial metaphases showing the radial configuration of acrocentric chromosomes in *C. flavicauda* (d) and *C. insolata* (e).

insolata and *C. flavicauda* chromosomes (Figure 3c, d). The fluorescent signals were lower under high stringency conditions (data not shown). The 5S rDNA clusters were
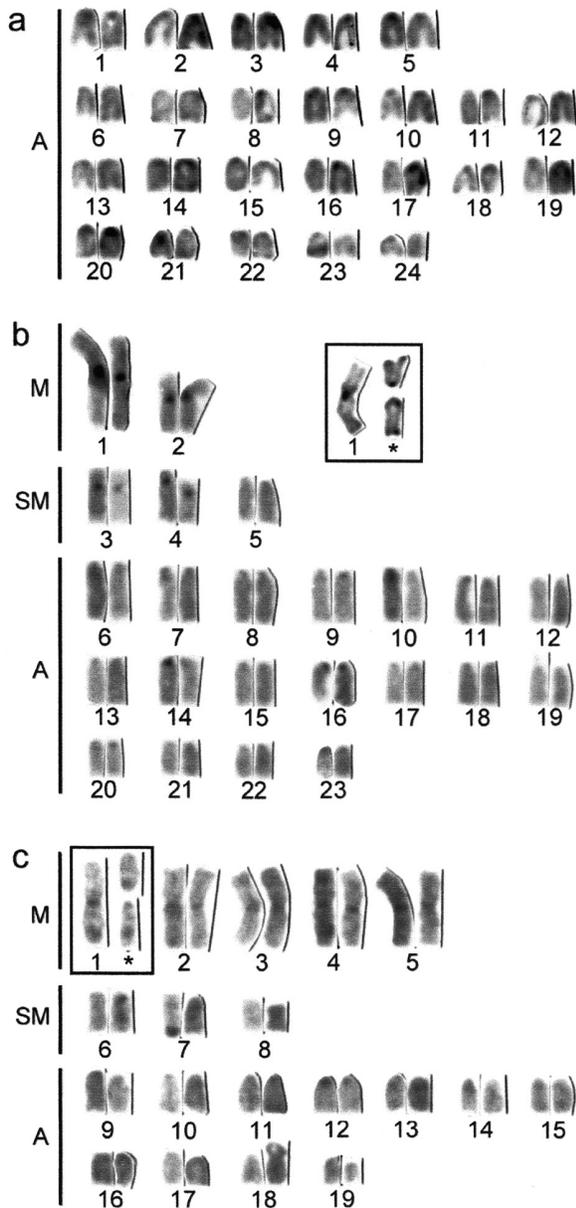
Figure 2 - C-banded karyotypes of *Chromis multilineata* (a), *C. insolata* (b) and *C. flavicauda* (c) with chromosomes related to centric fusion indicated in the boxes.

detected by FISH in the telomeric region of two large acrocentric chromosomal pairs in *C. insolata* (Figure 3e) and in the pericentromeric region of two large metacentric chromosomes, comparable to pairs 2 and 3, of *C. flavicauda* (Figure 3f).

Discussion

Robertsonian polymorphisms are important sources of karyotype diversification in several Perciformes groups, including the Pomacentridae (Ojima and Kashiwagi, 1981), Gobiidae (Thode *et al.*, 1985; Vitturi and Catalano, 1989;

Amores *et al.*, 1990; Caputo *et al.*, 1997) and Cichlidae (Martins *et al.*, 1995).

Most Pomacentridae species often show a numerically conserved karyotype of 48 chromosomes, with differences in the chromosome formula (FN = 48-90) probably being related to the occurrence of pericentric inversions during karyotype diversification (Takai and Ojima, 1987). In contrast, the Chrominae, which includes the genera *Acanthochromis*, *Azurina, Chromis* and *Dascyllus*, shows a particular trend towards Robertsonian polymorphisms. The diploid number can vary between 2n = 47-48 in *Dascyllus trimaculatus*, 2n = 34-37 in *D. reticulatus*, and 2n = 27-33 in *D. aruanus* (Ojima and Kashiwagi, 1981). Asynchronic hermaphroditism is common among these fishes and the likelihood that these divergent cytotypes are associated with multiple sexual chromosomes can be excluded (Ojima and Kashiwagi, 1981).

Fish species of inter-oceanic distribution, from the Caribbean reefs and from the Gulf of California in the Pacific Ocean, had a Pleistocene radiation (Shulman and Bermingham, 1995; Ward, 1995), indicating that the changes which occurred in this period were drastic enough to affect distinct groups with wide or narrow migratory capacities. At the beginning of the Pleistocene 700,000 years ago, the South American continent suffered a depression and the ocean overflowed, a condition that persisted throughout the Quaternary. Historic oceanographic events (1-5 MY) appear to have produced behavioral isolation that has affected the evolution and biogeographical patterns of some fish populations along the Brazilian coastline (Goodbred and Graves, 1996). During periods of regression, sequential bottleneck populations may have been generated by a high loss of habitats (Shulman and Bermingham, 1995; Ward, 1995), thus contributing to the current differentiation along the coastline.

Previous studies of the genus *Chromis* revealed that *C. chromis* had 2n = 48 and FN = 48 (Alvarez *et al.*, 1980), *C. chrysura* had 2n = 48 and FN = 50, and *C. caerulea* had 2n = 48 and FN = 48 (Ojima and Kashiwagi, 1981), indicating the occurrence of a highly conserved karyotype among these fishes. The reduction in chromosome number and the presence of distinctive metacentric chromosomes in *C. insolata* and *C. flavicauda* may have resulted from centric fusions. Such polymorphism could be transitory and represent a step in an evolutionary process such as karyotypic orthoselection (*sensu* White, 1973) among Chrominae species.

The radial chromosomal configurations observed in several metaphases of *Chromis* have also been reported in other fish species (Aguilar *et al.*, 1998). This configuration may be the remains of a later arrangement of these chromosomes during the preceding interphase, which could have facilitated chromosomal exchange among non-homologous chromosomes (Aguilar *et al.*, 1998).
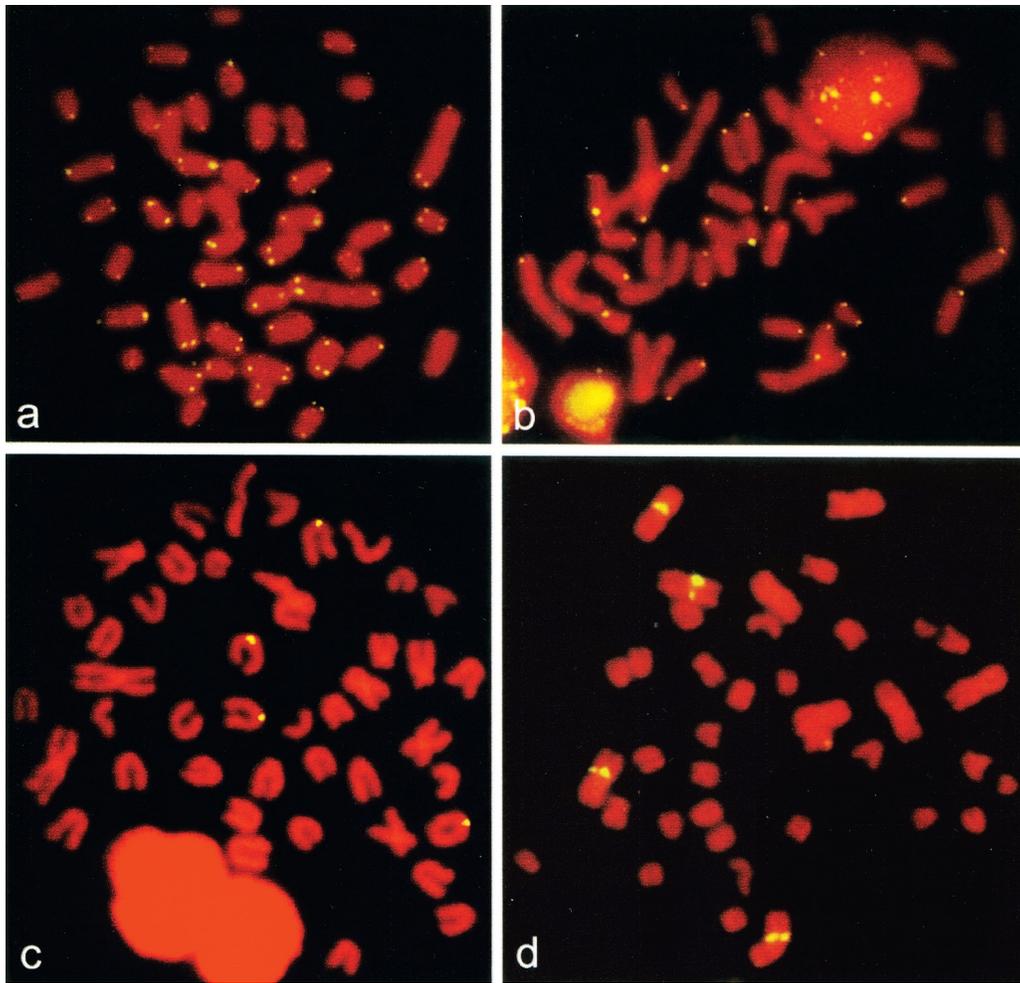
Figure 3 - FISH using a telomeric sequence probe in *C. insolata* (a) and *C. flavicauda* (b). The results obtained using a 5S rDNA probe in these same species are shown in (c) and (d), respectively.

The NOR distribution at the end of acrocentric chromosomes in *C. flavicauda* and *C. insolata* was quite similar to the distribution pattern observed in other Chrominae species (Takai and Ojima, 1995). The detection of NOR sites in non-homologous chromosomes in the studied species may also indicate increased plasticity of the chromosomal complement of these fishes, resulting in extensive polymorphism such as described in this study.

The pronounced conservation of telomeric sequences among vertebrates (Meyne *et al.*, 1989) was further demonstrated by the positive hybridization of the *Xenopus* telomeric probe with the chromosomes of *C. flavicauda* and *C. insolata*. Internal telomeric sites were not observed in either species, regardless of the stringency conditions for hybridization. The failure to detect internal telomeric sequences does not necessarily refute the hypothesis of centric fusions in the karyotype of these species. Telomeric sequences could have been eliminated by subsequent modifications or may be too small to be detected by cytogenetic methods (Meyne *et al.*, 1990; Nanda *et al.*, 1995). In some cases, telomeric sequences can be interspersed with repeti-

tive DNA regions (Garrido-Ramos *et al.*, 1998) and can occur within or near heterochromatic blocks (Meyne *et al.*, 1990; Abuín *et al.*, 1996). The absence of interstitial heterochromatic blocks in *Chromis* could account for the lack of ectopic sequences in fused chromosomes.

In contrast to the telomeric sequences, the FISH experiments based on 5S rDNA in *C. flavicauda* and *C. insolata* corroborated the hypothesis of centric fusion in the genus *Chromis*. In both species, the 5S and 45S rDNA loci occurred on different chromosomes. This is the condition most frequently observed in fish and reflects the independent evolution of distinct genes subjected to diverse selection pressures (Martins and Galetti, 1999). The interstitial location of 5S rDNA sites on two metacentric pairs (pairs 2 and 3) of *C. flavicauda* may be indicative of a sequential stage of non-homologous fusion involving acrocentric chromosomes of *C. insolata*. Whether these genes were actively involved in fusion events remains unknown. However, the current chromosome mapping of 5S rRNA genes reinforces the hypothesis that Robertsonian rearrangements

have occurred during karyotype diversification in this group of fish.

Acknowledgements

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