Constitutive heterochromatin and nucleolus organizer region in the knifefish, *Apteronotus albifrons* (Pisces, Apteronotidae)[1]

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Summary. The C-banding and silver staining of the chromosomes of the knifefish *Apteronotus albifrons* (2n = 24), demonstrated the presence of constitutive heterochromatin in the centromeric region of every chromosome, except pair 4, where the entire long arm was darkly stained, the silver stain positive nucleolus organizer region (NOR) being embedded in it.

The use of banding techniques in fish chromosomes is still rarely described in the literature. Results with C-banding and silver staining have been reported for some species[2–9], and show that banding techniques may not only help in the understanding of the structure of fish chromosomes, but may also have cytotaxonomic value[9].

We present here the analysis of the chromosomes of *Apteronotus albifrons,* with respect to the constitutive heterochromatin distribution and nucleolus organizer region (NOR) location. This fish has a karyotype consisting of 2n = 24 chromosomes as described earlier by Howell[10].

9 specimens (7 males and 2 females) of *Apteronotus albifrons* (Linnaeus, 1766) from Marajó Island (Pará, Brasil), were used. Chromosome preparations were made from gills and kidneys, following the method of Bertollo et al.[11]. The staining of the constitutive heterochromatin was performed according to the method of Sumner[12], and the NOR staining followed the technique described by Bloom and Goodpasture[13], as modified by Lau et al.[14].

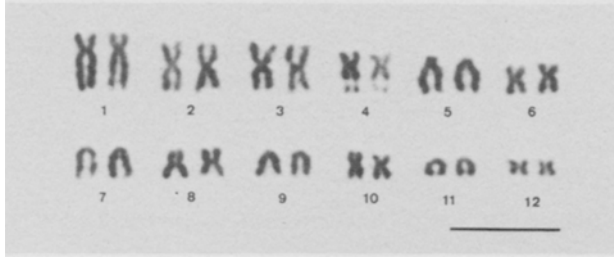

Figure 1. Karyotype of a metaphase cell of gill epithelium of a male knifefish, *A. albifrons* (bar 10 μm).

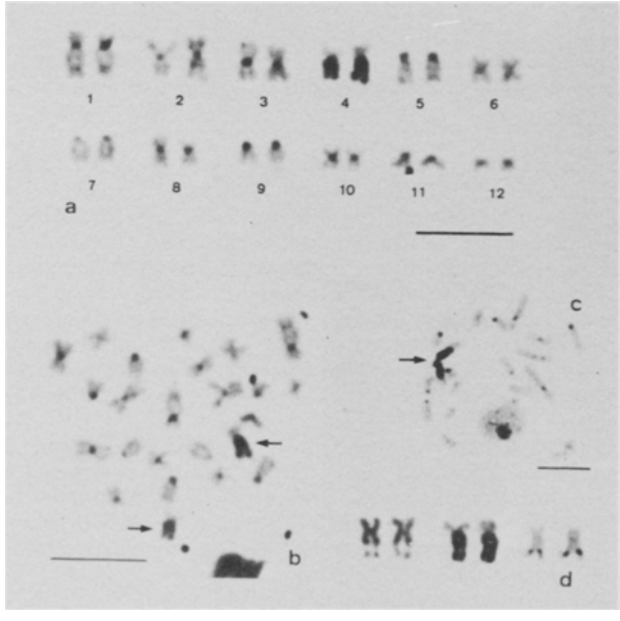

Figure 2. C-banded chromosomes from kidney cells of *A. albifrons* (bar 10 μm). *a* Karyotype. *b* A metaphase chromosome spread. The arrows point to the chromosomes of pair 4. *c* A metaphase showing pair 4 in association (arrow). *d* Secondary constriction of pair 4: normal Giemsa staining, C-banding and silver staining.

The diploid number was 2n = 24 chromosomes and no numerical or morphological differences were observed between males and females. The karyotype of a male is presented in figure 1. Chromosomes were arranged in order of decreasing size and classified, according to the position of the centromere, into: 6 pairs of metacentrics (pairs 2, 3, 6, 8, 10 and 12); 2 pairs of submetacentrics (pairs 1 and 4); 1 pair of subtelocentrics (pair 5) and 3 pairs of acrocentrics (pairs 7, 9 and 11). Pair 4 contained an interstitial secondary constriction in the long arm and, in many cases, this homologous pair was seen in association (figs 2c and 3b).

All chromosomes of *A. albifrons* (fig. 2b) presented heterochromatin in the centromeric regions. The C-bands in chromosomes 1, 3, 5, 8, 9 and 10 were very intense. Chromosomes 2, 7 and 11 presented a less intense C-band, and chromosomes 6 and 12 had very faint bands that usually could hardly be seen. The long arm of chromosome 4 was C-band positive with a thin, less-stained interstitial region that seems to correspond to the secondary constriction (fig. 2d).

With silver staining (fig. 3a), 2 darkly stained NORs were seen in the long arm of each of the chromosomes of pair 4, in the region corresponding to the secondary constriction.

A. albifrons presents a very peculiar karyotype, because it has a very low diploid number and most of its chromosomes can be identified in conventionally stained preparations.

The karyotype of *A. albifrons* was first described by Howell[10]. The diploid number was the same, but the author described 7 pairs of metacentrics and 1 pair of submetacentrics; we found, instead, 6 pairs of metacentrics and 2 pairs of submetacentrics. Misinterpretation may have occurred

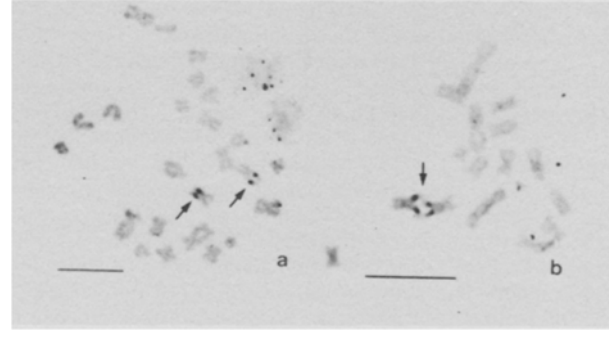

Figure 3. Silver stained chromosomes from gill epithelium of *A. albifrons* (bar 10 μm). *a* A metaphase spread showing Ag-NORs (arrows). *b* A partial metaphase showing pair 4 in association (arrow).

because, sometimes, the secondary constriction of pair 4 cannot be well observed and the chromosomes may be considered as metacentrics.

The different patterns of C-bands associated with the morphological differences of the chromosomes allowed a precise identification of all the chromosome pairs. The distribution of the constitutive heterochromatin associated with the NOR observed in the long arm of pair 4 is different from that described for another fish, *Umbra limi*, where sequential banding analysis showed that the silver-stained region was also C-band positive[3]. In *A. albifrons*, however, the NOR is not C-band positive, as shown in figure 2d. This situation is very similar to mammalian species where NORs have never been found to be C-band positive[15].

Most of the available data in the literature[3,7,9] and unpublished data of the present authors, involving 3 more species of Gymnotiformes, show that in many fishes a NOR seems to exist in only 1 pair of homologues; in some cases there is a suggestion of the involvement of more than 1 chromosome pair in the nucleolar organization, either by the presence of tiny dark areas over another chromosome pair[2], or by the indication that the silver-stained chromosomes were not apparently homologues[8]. These facts lead us to speculate that in fishes the usual condition could be the presence of only one or a few chromosome pairs involved in the nucleolar organization. Inversion and translocation mechanisms could explain the different morphology of the NOR bearing pair even in closely related species, as is the case in the Anostomidae[9].

The identification of all the chromosome complement of *A. albifrons* by banding procedures may help in the studies of the cytotaxonomy and evolution of the order Gymnotiformes, and also in experimental studies involving fish chromosomes.

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Effect of progesterone on the formation of the ovipositor in female bitterlings (*Rhodeus sericeus amarus* BLOCH, 1782) (Teleostei, Cyprinidae)[1]

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Summary. Female bitterlings *(Rhodeus sericeus amarus)* were treated with the sexual hormone progesterone at a dose of 170 µg/l for 2 weeks. Ovipositors from 1–4 centimetres in length grew out in all females within 24 h. The ovipositors were studied by electron microscopy (SEM, TEM).

The bitterling *Rhodeus sericeus amarus* is a small European fresh-water fish with remarkable breeding habits. During the spawning period the female developes an ovipositor from which eggs are injected into the inhalent siphon of a mussel of the genera *Anodonta* or *Unio*.

The purpose of this paper is to clarify whether or not progesterone can induce the development of an ovipositor and/or control its appearance. Female bitterlings lacking ovipositors were treated with the female sex hormone progesterone and then checked for the occurrence of egg depositors. The induced ovipositors were examined ultrastructurally.

24 female *Rhodeus sericeus amarus* were placed in 3 60-l aquaria (8 per tank). 1 60-l tank was used as a control aquarium containing 6 bitterlings. The hormone progesterone was dissolved in 96% ethyl alcohol at a concentration of 10 mg/ml. This solution was added to the 3 aquaria at a dose of 1 ml/60 l of aquarium water, creating a concentration of 170 µg of progesterone/l[2]. The control aquarium was given a dose of 1 ml of 96% ethyl alcohol/60 l of water. The fish were treated for 2 weeks.

The treated females were anesthetized with 0.5–1% Anestesin (=4-aminobenzoic-acid-ethylester, Serva). The ovipositors were then cut off with fine scissors and placed in 5% glutaraldehyde buffered with veronal acetate and phosphate buffers pH 7.4, 4 °C for 2–10 h. After washing with adaequate buffers (3 times) ovipositors were postfixed in 2% osmium tetroxide, dehydrated in a series of ascending alcohols and embedded via propylene oxide in Vestopal W and Araldite. Ultrathin sections were cut with a Reichert Om U 3, double-stained in uranyl acetate and phosphotungstic acid en bloc[3] or in uranyl acetate and lead citrate[4]. They were then viewed in a Zeiss EM 9S and a Philips EM 400.

1 of 30 females treated with 170 µg of progesterone/l aquarium water died during the 1st week of testing and 2 died during the 2nd week. In all females ovipositors were observed within 24 h, the length ranging from 1 to 4 cm. The 6 control females showed no sign of an ovipositor during the 2-week testing period.

Ovipositors can also be induced by addition of 1–2 ml urine of gravid women/60 l aquarium water (Riehl, unpublished). After this treatment only 80% of the female bitterlings show ovipositors, which also grow out within 24 h. The induction of ovipositors by treatment with the urine of gravid women was formerly employed in diagnosis of