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Karyotypic characterization of bream, *Abramis brama* (Pisces, Cyprinidae)

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A b s t r a c t . The karyotype of bream *Abramis brama* was analysed by means of C-banding, replication banding, DAPI fluorescent staining and *in situ* hybridisation with 18S rDNA and telomeric probes. The use of the *in vivo* 5-bromodeoxyuridine incorporation technique enabled the induction of replication bands of the RBA type in the karyotype. C-bands corresponded to late replicated chromosome regions. 18S rDNA clusters were found on one chromosome pair. Telomeric sequences were observed only on ends of chromatids. The karyotype morphology and NOR phenotype of *A. brama* are very similar to those found in other species of the leuciscine cyprinids karyotyped so far.

Key words: chromosomes, Leuciscinae, rDNA, replication, telomere

Introduction

The bream *Abramis brama* (Cyprinidae, Cypriniformes) is one of the most abundant fish species in central Europe and Asia. Data on its chromosome complements have been reported by several authors (N y g r e n et al. 1975, B a r s h e n e 1977, B a r s h e n e et al. 1983, A r e f j e v & K a r n a u c h o v 1989). All these karyotype studies have been based on conventionally Giemsa-stained chromosomes. This paper is a continuation of our previous investigation on *Abramis brama* from Poland (J a n k u n et al. 1997). The aim of the present study was to describe the bream karyotype by means of replication, C-banding and molecular cytogenetics methods.

Materials and Methods

Nineteen adult bream (*Abramis brama*): 8 females and 11 males were caught with gill nets in Lake Kortowo in Olsztyn, Poland. Chromosome preparation was done according to R á b & R o t h (1988) with modifications described by J a n k u n et al. (1997). Replication banding followed the method described by J a n k u n et al. (1998). *In vivo* BrdU (Sigma) incubation equalled 6 h. C-banding was performed according to S u m n e r (1972) with alkaline denaturation at 50 °C for 2 minutes. *In situ* hybridization (ISH) using as a probe a plasmid including rDNA transcription unit and most of the 18S rRNA gene (pB18') was done as described previously (J a n k u n et al. 2001). Chromosomes were stained with 4', 6-diamidino-2-phenylindole (DAPI) using antifade (Vectashield Vector, Burlingame, USA) with DAPI. The telomere PNA probe (synthetic DNA/RNA analogue) conjugated

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with FITC was used in a two hour – long hybridization reaction, performed according to the manufacturer's instructions (DAKO, Denmark) (O c a l e w i c z et al. 2003). The chromosomes were photographed with an Optiphot 2 (Nikon) microscope and digital camera Coolpix 950.

Chromosomes were classified according to the system of L e v a n et al. (1964).

Results and Discussion

The karyotype of bream revealed in our study consisted of 7 pairs of metacentric chromosomes, 11 pairs of submetacentric and 7 pairs of acrocentric chromosomes (Fig. 1). Such a karyotype seems to be typical for representatives of the Leuciscinae subfamily, which are characterized by 2n=50 and with the uniarmed chromosome pair as the largest element of the complement easily recognizable (V a s i l' e v 1985, R á b & C o l l a r e s - P e r e i r a 1995). Some other cyprinid genera, such as *Leuciscus*, *Blicca*, *Alburnus*, *Phoxinus*, *Chondrostoma* and *Eupallasella*, belong to the Leuciscinae subfamily according to H o w e s (1991) and C o b u r n & C a v e n d e r (1992). The karyotypes of Leuciscinae

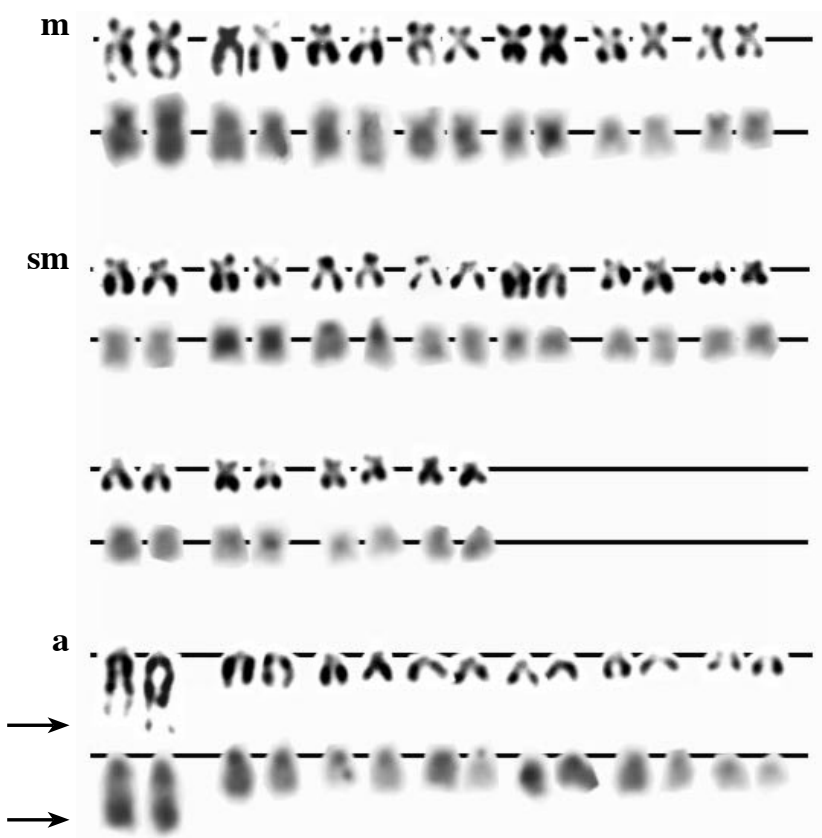

Fig. 1. Karyotype of bream *Abramis brama*. Homologues were identified by replication bands (upper rows) and C-banding (lower rows). Arrows indicate C-positive and late replicating heterochromatin of the first acrocentric chromosome pair, marker chromosome for leuciscine.

are composed mainly of biarmed elements, suggesting a high evolutionary position among other Cyprinidae (Vasiľev 1985, Ráb & Collares-Pereira 1995). Also the location of nucleolar organizer regions (NORs) is similar among species of this group. Most other European cyprinid fish species, as well as most of the North American species, have one pair of NORs (Takai & Ojima 1986, Amemiya et al. 1992, Birstein & Vasiľev 1987). However, several species with two pairs of NORs are known: *Eupallasella perenurus* (Boroń et al. 1997, Boroń 2001), *Chondrostoma lusitanicum* (Rodrigues & Collares-Pereira 1996, Collares-Pereira & Ráb 1999), *Phoxinus phoxinus* (Boroń 2001).

Gold & Amemiya (1986) and Amemiya & Gold (1988) have hypothesized that the single NOR located terminally on acrocentric chromosome represents the plesiomorphic state for Cyprinidae. Two or multiple pairs of chromosome bearing rDNA sites seem to represent a derived condition among Cyprinidae as well as among Leuciscinae (Rodrigues & Collares-Pereira 1996, Collares-Pereira & Ráb 1999, Boroń 2001).

Examination for nucleolar organizer regions (NORs) using *in situ* hybridisation with 18S rDNA probe revealed their location in the whole of the short arm of small subtelocentric chromosome pair (Fig. 2a). This supports our previous data obtained by means of indirect methods such as chromomycin A_3 and silver nitrate stainings (Jankun et al. 1997).

Chromosome banding techniques have been applied to the chromosomes of European cyprinids in only a small number of investigations (Klinkhardt et al. 1995). In the present study C-banding showed a very low content of heterochromatin in chromosomes, except for the heterochromatic block in the largest chromosome pair which is acrocentric and pericentromeric blocks in some biarmed chromosome pairs (Fig. 1). Rather low heterochromatin concentrations in the chromosomes of two minnow fish, *Phoxinus phoxinus* and *Eupallasella perenurus* have been described (Boroń 2001). The C bands occurred in the NOR-sites and in the centromere regions of uniarmed and some biarmed chromosomes.

Replication banding enabled identification of early and late replicating regions in chromosomes. Heterochromatic C-positive regions, which are build of repetitive DNA fragments and are not transcriptionally active (Sumner 1998), were replicated at the end of the DNA synthesis phase. C-banded heterochromatin was found to be greatly concerned with the karyotype evolution in bitterlings (Cyprinidae) (Ueda et al. 2001). Scarce data on banding study in leuciscine cyprinids prevent comparative analyses. The replication banding was successfully used for comparing roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophthalmus*) (Cyprinidae) karyotypes, which are identical on Giemsa staining but different after BrdU treatment (Hellmer et al. 1991).

Investigation by means of fluorochrome DAPI did not reveal any specific AT-rich heterochromatin (not shown). Similar results were obtained in another cyprinid *Tinca tinca* (Mayr et al. 1986). It could be the next common karyotype feature not only for Leuciscinae, but also for the fish from the family Cyprinidae.

PNA FISH enabled recognition of all conservative sequences $(TTAGGG)_n$ on *Abramis brama* chromosomes. All chromosomes of the complement had positive signals at the ends of chromatids. No interstitial signals were observed (Fig. 2b). The interstitial sites of the (TTAGGG)n telomeric sequence (ITS) were found in few fish species (Reed & Phillips 1995, Salvadori et al. 1995, Abuin et al. 1996) and they could be remnants of chromosome rearrangements that occurred in the course of karyotype

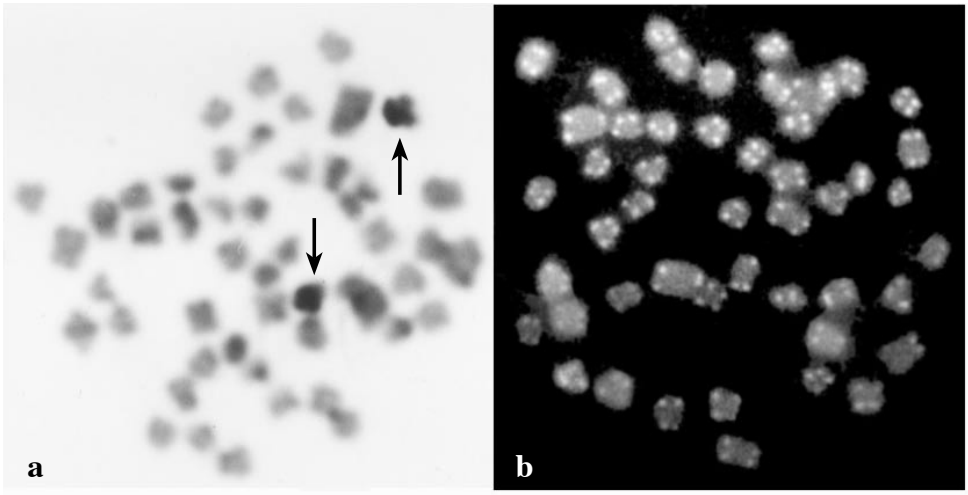

Fig. 2. Metaphase chromosome of bream *Abramis brama*, a – after *in situ* hybridization (ISH) with a plasmid containing 18S rRNA gene (pB18'), b – after telomere PNA fluorescence *in situ* hybridization. Arrows indicate 18s rDNA location.

evolution. The ITS seem to be destabilizing elements in chromosomes and the correlation between these sites and chromosome rearrangements are proved (B o u f f l e r 1998). There is a hypothesis that ITS enable greater flexibility for the karyotype change (M e y n e et al. 1990).

In conclusion, the karyotype and NOR phenotype of *A. brama* is very similar to that found in the other species of leuciscine cyprinids karyotyped so far. The chromosome banding patterns may be useful in further studies of this fish group.

A c k n o w l e d g m e n t s

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