Marine Biology 112, 37–41 (1992)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Karyotype analyses reveal inter-individual polymorphism and association of nucleolus-organizer-carrying chromosomes in *Capros aper* (Pisces: Zeiformes)

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Date of final manuscript acceptance: September 19, 1991. Communicated by M. Sarà, Genova

Abstract. Three different karyomorphs with $2n = 46$, $2n = 44$ and $2n = 42$ for *Capros aper* (L., 1758) (Zeiformes) collected from the Gulf of Lion, near Banyuls-sur-Mer, France, in July 1990 were determined. Karyomorphs were characterized by the same arm number [Fundamental number (FN) = 50], suggesting that chromosome variations are due to Robertsonian fusions. In somatic metaphase spreads stained with silver nitrate, nucleolus organizer regions (NORs) consistently occupied a terminal position on the short arms of two small submetacentric chromosomes. Ca. 30% of silver-stained metaphases in each specimen showed NOR chromosomes associated in pairs by their nucleolus organizer regions.

Introduction

The order Zeiformes includes four families: Zeidae, Grammicolepididae, Oreosomatidae and Caproidae. In the Mediterranean Sea, the family Caproidae, represented only by *Capros aper* (Whitehead et al. 1986), has remained unexplored cytogenetically.

The present paper describes the *Capros aper* karyotype, non-differentially stained with Giemsa for conventional analysis and with silver nitrate for the characterization of nucleolus organizer regions (NORs). Inter-individual chromosome polymorphism and observed associations of nucleolus organizer chromosomes are discussed.

Materials and methods

Capros aper, which ranges in size from 5 to 13 cm, inhabits mainly depths from 100 to 400 m (Whitehead et al. 1986); hence, specimens in a healthy condition are seldom obtained. In four consecutive expeditions during July 1990, seven sexually immature individuals were captured by seine in the Gulf of Lion, near Banyuls-sur-Mer, France. They were identified according to the guidelines of Whitehead et al. (1986).

Each specimen was injected intraperitoneally with colchicine (0.1%, 1 ml/30 g body weight) and sacrificed 2 h later. Kidney and spleen tissues were removed and minced in 0.075 *M* KCl for 20 min. The suspension was centrifuged, supernatant discarded and the cell pellet fixed in two changes of fresh methanol-acetic acid fixative (3:1). Drops of cell suspension were then placed on clean slides at 0 °C.

Slides were stained with 5% Giemsa, pH 6.8, for conventional analysis. After destaining in absolute ethanol, these slides were treated following the controlled silver staining technique of Howell and Black (1980) for localization of the NORs.

Observations and photomicrographs were made using a Jenamed 2 light microscope. Chromosomes were classified according to Levan et al. (1964).

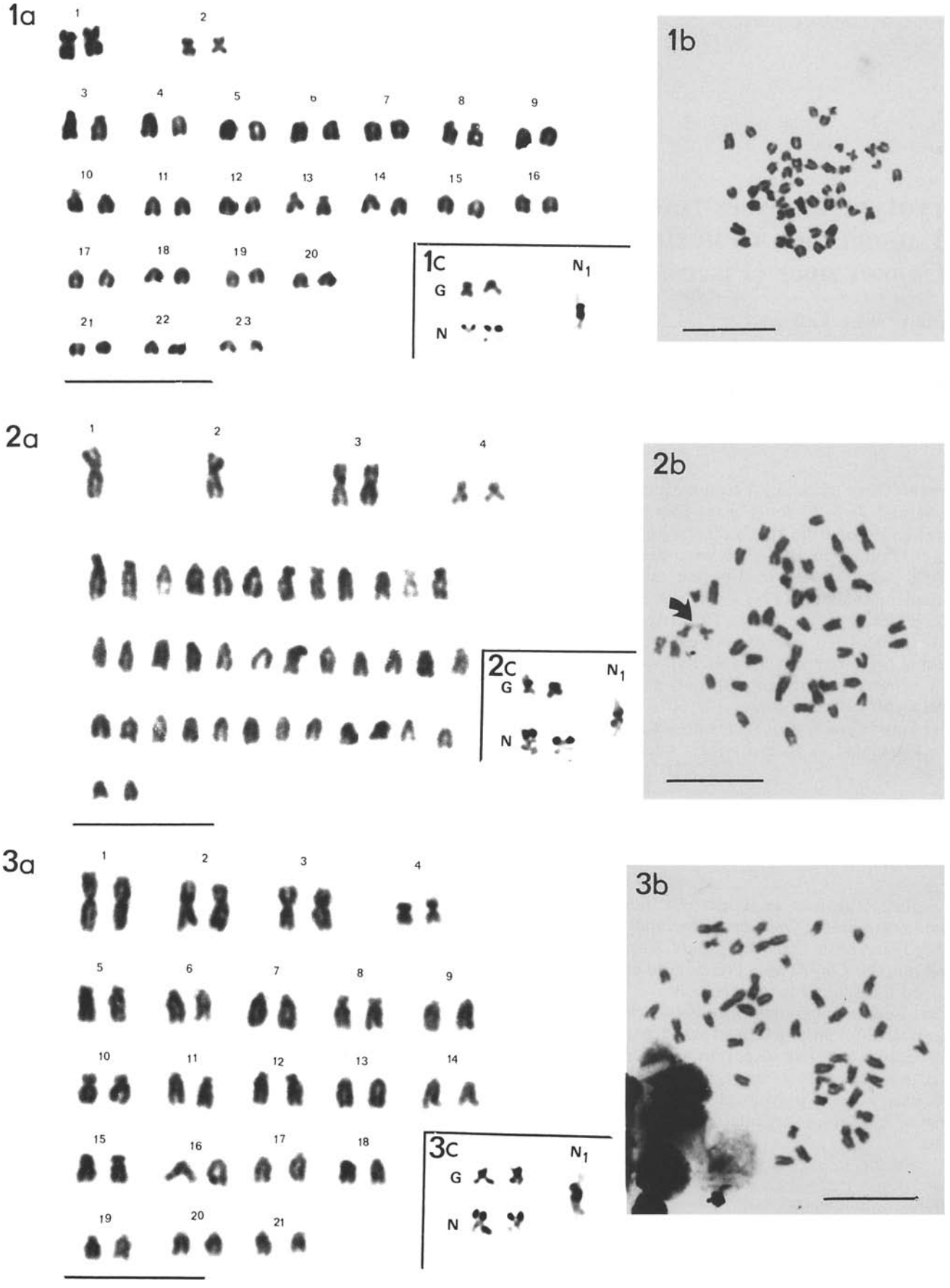
Results

Microscopic analysis revealed that preparations from four *Capros aper* individuals had enough spreads for chromosome study. Based on 30 somatic metaphase spreads per specimen, three karyomorphs with different diploid numbers were found: A-karyotype ($2n = 46$) (one specimen); B-karyotype ($2n = 44$) (one specimen); and C-karyotype ($2n = 42$) (two specimens).

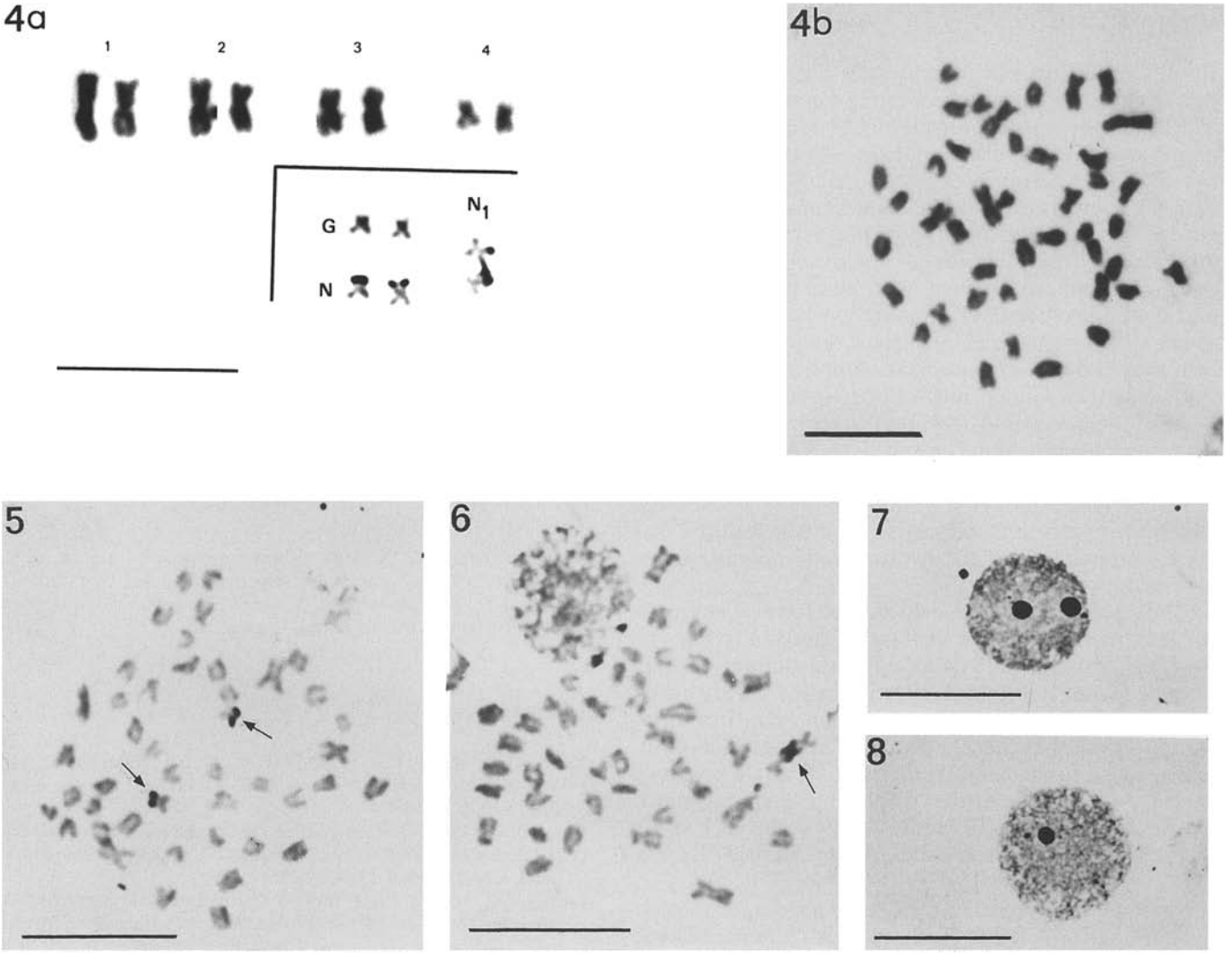
Taking into account both morphology and size along with centromere position, the A-type complement could be arranged into 23 pairs (Fig. 1a). Pair 1 consisted of large metacentrics; Pair 2 was submetacentric; Pairs 3, 8, 10 and 13 were subtelocentric, and all other pairs were composed of acrocentric chromosomes. The fundamental number (FN) was 50.

The B-karyotype (Fig. 2a) differed from the A-type by including two large bi-armed elements. Furthermore, the absence of four mono-armed elements was observed; thus the fundamental number was kept constant (FN = 50). Due to slight differences in both morphology and size, chromosome nos. 1 and 2 in Fig. 2a might be non-homologues; hence, we preferred to arrange the mono-armed chromosomes by decreasing length.

The C-karyotype displayed six large metacentrics which could be arranged into pairs, and two small submetacentrics designated as Pair 4; moreover, eight subte-



Figs. 1–3. *Capros aper*. Fig. 1. (a) Karyotype from fish no. 1 ($2n=46$); (b) metaphase plate; (c) NOR chromosomes (G = Giemsa-stained, N = silver-stained, N_1 = NOR chromosomes associated in pair). Fig. 2. (a) Karyotype from fish no. 2 ($2n=44$); (b) metaphase plate (end-to-end association indicated by arrow); (c) NOR chromosomes. Fig. 3. (a) Karyotype from fish no. 3 ($2n=42$); (b) metaphase plate; (c) NOR chromosomes. All scale bars = 10 μm



Figs. 4–8. *Capros aper*. Fig. 4. (a) Metacentric and submetacentric pairs from fish no. 4 ($2n=42$); (b) metaphase plate; (c) NOR chromosomes (G = Giemsa-stained, N = silver-stained, N_1 = NOR chromosomes associated in pair). Fig. 5. Silver-stained metaphase from fish no. 2; NOR chromosomes indicated by arrows are not associated. Fig. 6. Silver-stained metaphase from fish no. 2; the association of NOR chromosomes is indicated by arrow. Fig. 7. Silver-stained interphase nucleus with two silver areas. Fig. 8. Silver-stained interphase nucleus with one silver area. All scale bars = 10 μm

locentrics and 26 acrocentrics could be detected (Fig. 3a). Due to the fact that the increase in metacentrics was accompanied by a parallel decrease in the number of acrocentrics, the fundamental number remained 50.

Thirty non-differentially stained mitoses from fish no. 4 were analyzed. The diploid number $2n=42$, and the presence of six large bi-armed elements occurring in pairs (Fig. 4a), allowed us to identify this complement as a C-type.

In three or four Giemsa-stained spreads out of the total of 30 analyzed per specimen, two small submetacentric chromosomes showed end-to-end association (e.g. see arrow in Fig. 2b).

After treatment with silver nitrate, all four *Capros aper* specimens displayed two small submetacentric chromosomes that were positively stained (Figs. 1c, 2c, 3c, 4c). This chromosome pair was identified as Pair 2 in the A-karyotype and as Pair 4 in both B- and C-karyotypes. The NORs were consistently located at the terminal position on the short arms of both homologues, which were distinct from each other in ca. 35 out of 50 spreads analyzed per specimen (Fig. 5, arrows) and seemed to be fused or associated in the remaining 15 spreads (Fig. 6, arrow; N_1 in Figs. 1c, 2c, 3c, 4c). None of these metaphases displayed chromosomes with additional structural alterations. Further, from analysis of the spreads without associated pairs we determined that the NORs were similar in size in three individuals (Figs. 2c, 3c and 4c) but different in all spreads of the fourth one (see N in Fig. 1c).

In addition, we examined the frequency of two (Fig. 7) or one (Fig. 8) silver-stained NOR in 100 interphase nuclei from one specimen; we found 58 nuclei with two NORs and 42 nuclei with one NOR, respectively.

Discussion

Results of the present investigation demonstrate variation in chromosome number among *Capros aper* individuals from the sea near Banyuls-sur-Mer, France, because three karyomorphs with $2n=46$, $2n=44$ and $2n=42$ were found. The occurrence of intrapopulational karyotype variability while FN values remained constant leads us to believe that a Robertsonian polymorphic system involving elements from the groups of subtelocentric or acrocentric chromosomes was present. Since the B-karyotype presumably includes two unpaired metacentric chromosomes which occur in pairs in the C-karyotype, it is evident that in the *C. aper* population polymorphism arose from at least two independent Robertsonian fusions. The B- and C-types would represent heterozygous and homozygous fusion events, respectively. Alternatively, if further studies using C- and G-banding procedures provide evidence in favour of non-homology of the large metacentric pairs in C-karyotype, it may be deduced that more than two fusions are involved in the polymorphism observed.

The diploid number $2n=46$ may be the original number for this species; nevertheless, the possibility that the bi-armed chromosomes of Pair 1 in this karyotype may be drived from Robertsonian translocation is very likely; hence, specimens without fusions might exist. It is interesting to note that those individuals having 48 chromosomes with a fundamental number of 50 would possess a karyotype very close to that displaying 48 entirely acrocentric chromosomes (FN = 48), widely recognized as the ancestral type within teleostean fishes (Ohno 1970, Chen 1971).

Similarly – as reported for *Gobius paganellus* (Vitturi et al. 1984, Thode et al. 1985) and for *G. fallax* (Thode et al. 1988), in which continuous karyotype variations ranging from $2n=48$ to 45 and from $2n=43$ to 38, respectively, have been found – *Capros aper* individuals with intermediate chromosome numbers such as $2n=45$ and $2n=43$ certainly existed in the population studied here. These values would originate by random combinations of gametes produced by the A- and B-type or by the B- and C-type specimens. We did not encounter these complements, due to the low number of individuals investigated.

Data from the literature indicate that intra-specific chromosome polymorphism of the Robertsonian type involving more than one chromosome pair is particularly widespread in the gobiid species *Gobius paganellus* (Vitturi et al. 1984, Thode et al. 1985), *G. fallax* (Thode et al. 1988) and *G. niger jozo* (Vitturi and Catalano 1989). Intra-specific polymorphism resulting from one fusion only has been described in salmonids (e.g. Thorgaard 1976, Hartley and Horne 1984 and references therein) as well as in other teleostean species (e.g. Ojima and Kashiwagi 1981, Vitturi et al. 1986, 1991, Vitturi 1988, Vitturi and Catalano 1988).

The finding of NORs in only one pair of homologous chromosomes in all four *Capros aper* specimens parallels the situation common to most teleostean fish analyzed so far; it suggests that constant numbers of active NORs per cell characterize this species. Nevertheless, size variation of the NORs was detected in one individual. Since in each metaphase the NORs of one chromosome were larger in size than those in the corresponding homologue, variation may represent a duplication pattern, thus causing a differential transcriptional activity in the ribosomal DNA (Miller et al. 1976). Polymorphism in NOR size is typical for many other fishes, e.g. the sparctic trout (Disney and Wright 1987), *Dicentrarchus labrax* and *D. punctatus* (Vitturi et al. 1990), *Uranoscopus scaber* (Vitturi et al. 1991) and several species of the order Gymnotiformes (Foresti et al. 1981) as well as of the family Anostomidae (Galetti et al. 1984).

Another finding in the present study was the non-random occurrence of metaphases with associated NOR chromosomes. The association of NOR-carrying chromosomes, at least among vertebrates, is a general phenomenon (Howell 1982, Babu and Verma (1985); it is interesting, however, that this event occurs with high frequency in a *Capros aper* population.

Acknowledgements. This research was supported by a grant from the Ministero della Pubblica Istruzione (Rome) and by "Laboratoire Arago", Banyuls-sur-Mer (France). The authors are indebted to Mr. D. Demma for his critical suggestions.

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