Heredity **67** (1991) 325–330

Received 11 January 1991

Intra-populational and intra-individual mosaicisms of *Uranoscopus scaber* L. (Perciformes, Uranoscopidae)

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Karyotypic analysis on 25 specimens of *Uranoscopus scaber* from the Gulf of Palermo has revealed the occurrence of an intra-populational mosaicism of Robertsonian type with three distinct diploid numbers: $2n = 30$, $2n = 29$ and $2n = 28$. Intra-individual mosaicism in the chromosome number ($2n = 30$ and 31 in two specimens and $2n = 28$ and 29 in one specimen) is also reported. One large metacentric pair is characterized by secondary constrictions, variable in length in different fish. Polymorphisms of these areas seem to have a genetic, rather than transcriptional, basis.

Keywords: karyology, mosaicism, pisces, *Uranoscopus scaber*.

Introduction

Although more than 200 species of teleostean Perciformes have been analysed cytogenetically (Sola *et al.*, 1981), many families of this order remain completely uncharacterized in this respect. One of these is the family Uranoscopidae, within which the genus *Uranoscopus* is represented by only one species in the Mediterranean (Whitehead *et al.*, 1986).

This paper analyses the chromosome constitutions of *Uranoscopus scaber* from the Gulf of Palermo and describes both intra-populational and intra-individual mosaicism in the chromosome numbers as well as structural polymorphism involving nucleolus organizer regions (NORs).

Materials and methods

Sixty-four specimens of *Uranoscopus scaber* caught by seine in the Gulf of Palermo and transported live to the Institute of Zoology, Palermo, Italy, were examined. All individuals were classified according to Whitehead *et al.* (1986) and sexed by examination of the gonads. Five specimens are now preserved in the Museum of the Institute of Zoology, University of Palermo.

Chromosome preparations were obtained by the standard air-drying technique applied to kidney and spleen tissues. Slides were stained with 5 per cent Giemsa pH 6.8, for conventional analysis. Nucleolus organizer regions (NORs) were characterized following the method of Howell & Black (1980); heterochromatic regions were revealed using the C-banding method of Sumner (1972); G-banding was performed according to Seabright (1971).

Observations and photomicrographs were made using a Jenamed 2 light microscope. Chromosomes were classified on the basis of the arm-length ratio (Levan *et al.*, 1964).

Results

Of the 64 specimens analysed in this study, only 25 produced preparations suitable for chromosome analysis. Counts of 20 spreads per specimen gave three distinct karyomorphs: with $2n = 30$ (three females, two males and nine sexually immature), $2n = 29$ (one female, one male and three sexually immature) and $2n = 28$ (two females, two males and two sexually immature). These karyomorphs, designated as A-, B- and C-type complements, had arm numbers (*FN*) of 56, 54 and 52, respectively.

The A-type complement (Fig. 1a) could be arranged in 15 pairs. The first nine were metacentrics; pairs 10 and 11 were submetacentrics, 12 and 13 were metacentrics and pairs 14 and 15 were telocentrics. Homologous chromosomes of pair 9 were characterized by secondary constrictions which differed in size. Two specimens, both possessing the modal diploid number

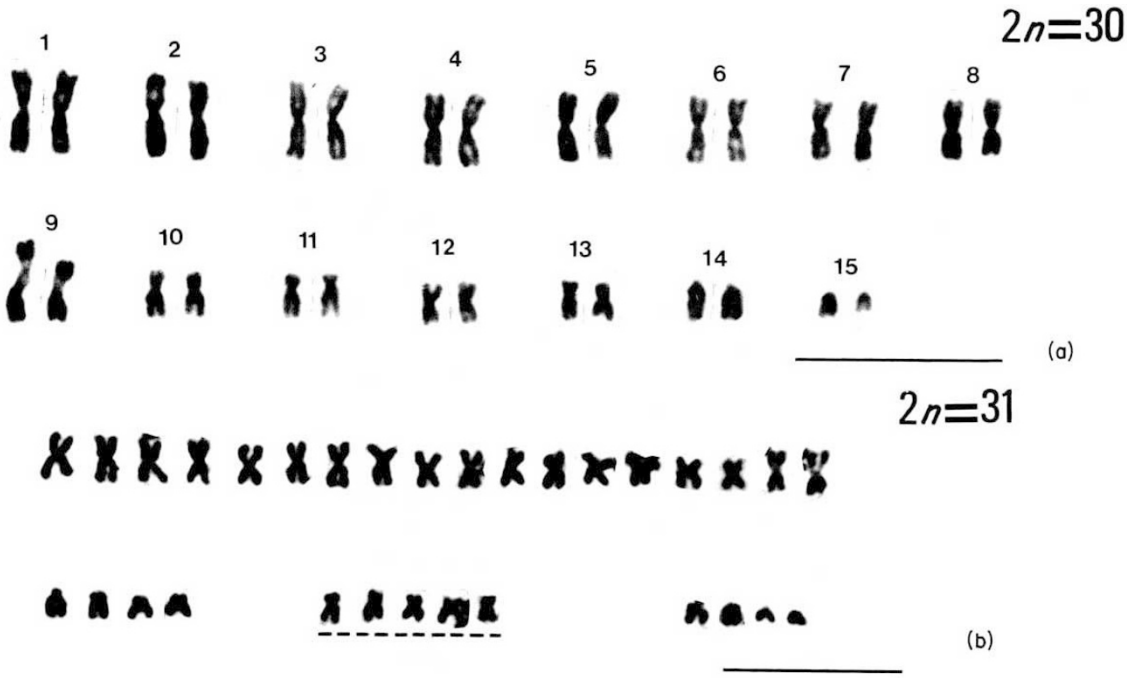

Fig. 1 (a) Giemsa non-differentially stained A-type. (b) Aneuploid spread ($2n = 31$) with chromosomes arranged in groups according to morphology and size.

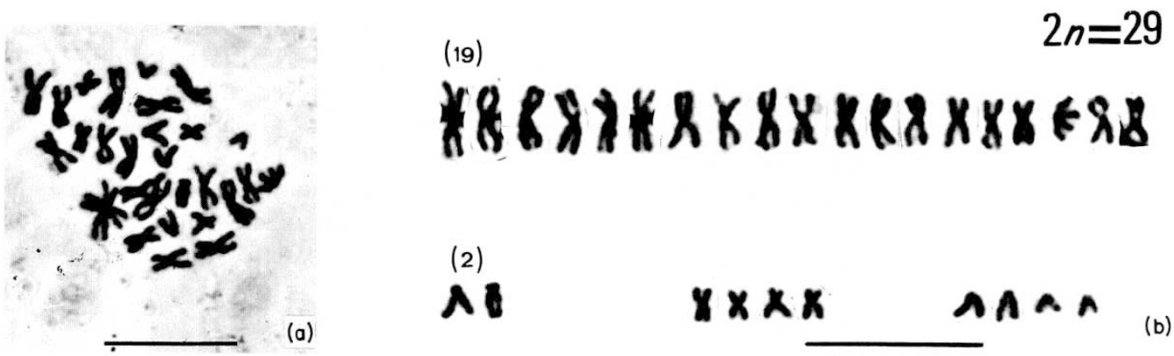

Fig. 2 (a) Mitotic metaphase spread with a modal diploid number of 29 and (b) chromosomes arranged in groups according to morphology and size (B-type).

of 30 chromosomes, had three and four (out of 20) cells respectively with $2n = 31$ (Fig. 2b); in both individuals an extra chromosome of the group of the medium-sized metacentrics (pairs 12 and 13) was observed.

The B-type (Fig. 2) consisted of 19 large metacentrics, two medium submetacentrics, four medium metacentrics and four telocentrics.

The C-type complement (Fig. 3a) differed from the A-type with respect to the presence of two large biarmed chromosomes derived from pairs 10 and 11. Homologous chromosomes of pair 9 had secondary constrictions which were both large in size. In addition, one individual, with the modal diploid number of 28 chromosomes, displayed three (out of 20) spreads with $2n = 29$ (Fig. 3b); in this case an extra chromosome belonged to the large metacentric group.

In silver-stained chromosome preparations, secondary constrictions of pair 9 were consistently involved in active nucleolus organization (Fig. 4a) and a correlation in dimensions between the NORs and these areas was observed (Fig. 4b). Secondary constrictions of chromosomes 9 appeared to be large in size in both homologues of six fish (Fig. 4a, see i), small in both homologues of four fish (Fig. 4b, see iii) and of different dimensions in 15 fish (Fig. 4c, see ii); in addition they showed a uniform length in all spreads of each individual.

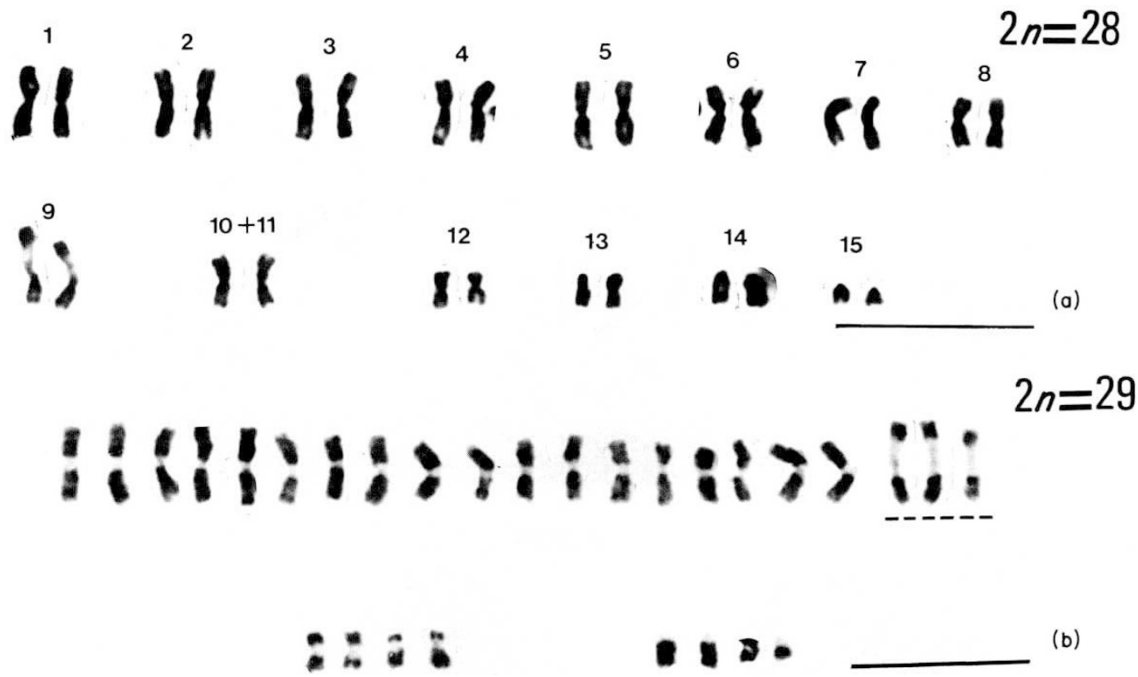

Fig. 3 (a) Giemsa non-differentially stained C-type. (b) G-banded aneuploid metaphase with $2n = 29$; chromosomes are arranged in groups according to morphology and size.

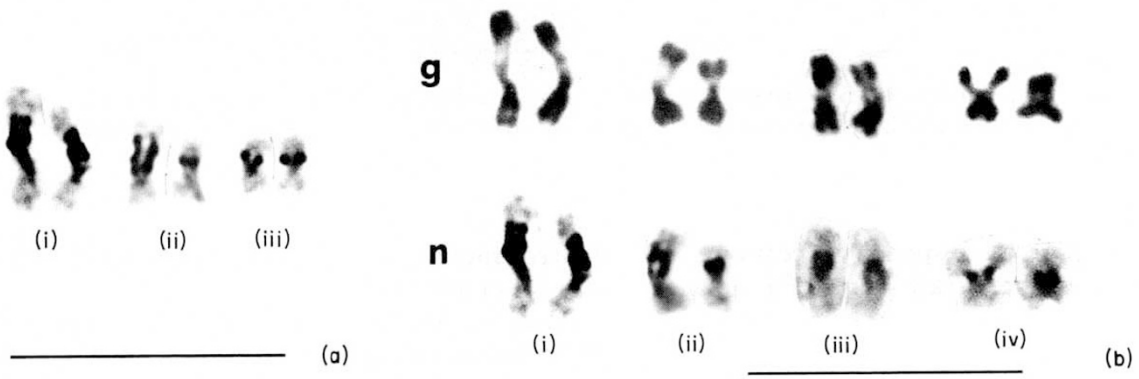

Fig. 4 (a) Silver-stained chromosomes with the NORs: (i) large in size; (ii) different in size, one large and one small; and (iii) small in size. (b) Pair 9 of different fish: g = giemsa stained and n = NOR stained.

After G-banding, large zones, which appeared light in staining, were observed in the centromeric regions of all chromosome pairs (Fig. 5a and b); also secondary constrictions of pair 9 were lightly stained throughout (Fig. 5a and b, see arrows). When the G-banding procedure was performed on the single specimen, which possessed cells with both 28 and 29 chromosomes, particularly interesting results were observed. Of 15 karyotyped spreads, 12 showed the modal diploid number of 28 chromosomes, while three had 29 chromosomes (Fig. 3b). In the latter, three elements showed a very large secondary constriction and a good correlation in their G-banding patterns (Figs 3b and 5c).

From C-banding analysis, conspicuous heterochromatic bands of approximately the same dimensions were observed and located in the centromeric regions of all chromosome pairs of both A- and C-types (Fig. 5d and e). Secondary constrictions of pair 9, which appeared moderately C-positive, were distin-

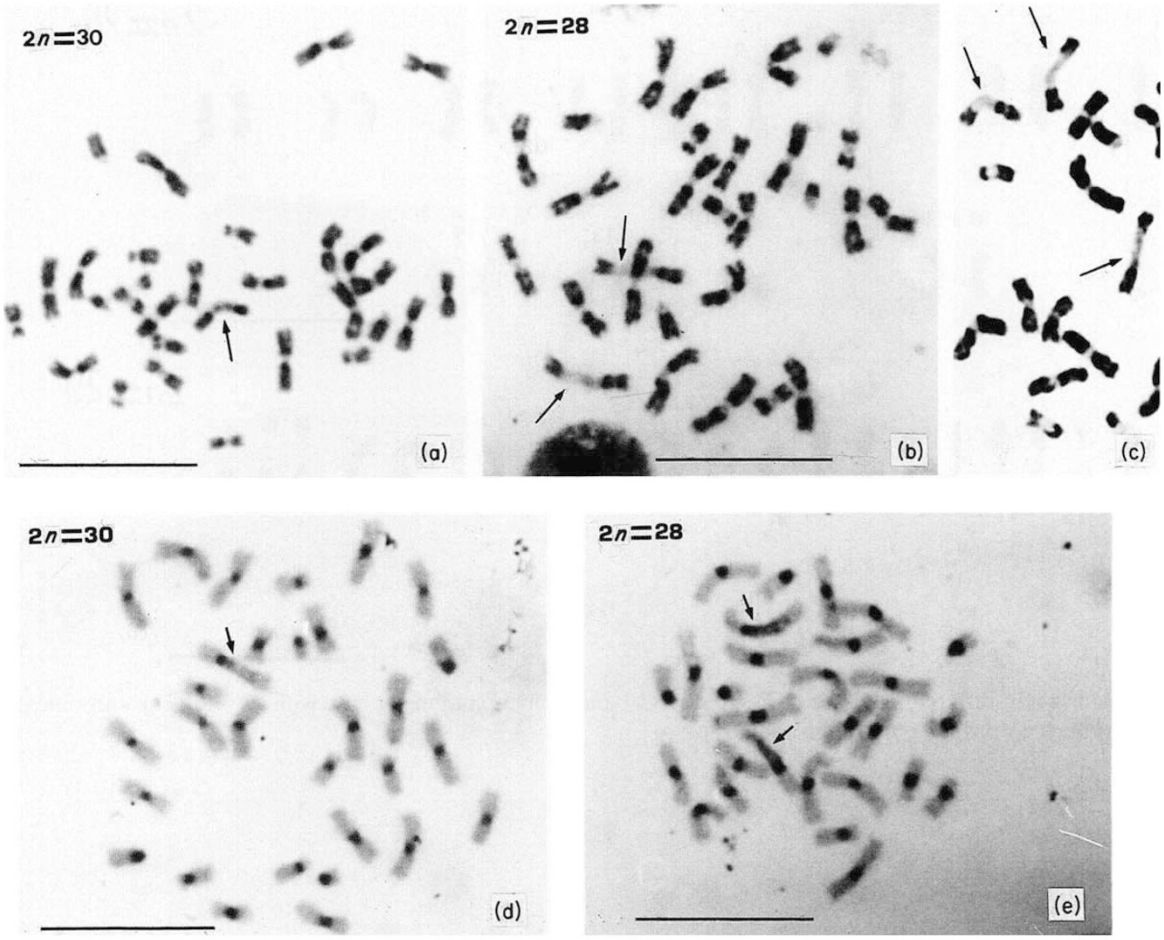

Fig. 5 (a) G-banded spread with $2n = 30$. (b) G-banded spread with $2n = 28$. (c) Partial G-banded aneuploid spread ($2n = 29$) (arrows indicate three elements each with a large secondary constriction). (d) C-banded spread with $2n = 30$. (e) C-banded spread with $2n = 28$.

guishable from the corresponding centromeric C-bands which were more darkly stained (Fig. 5d and e, see arrows).

Discussion

Karyotypic variability in the chromosome number ranging from $2n = 28$ to $2n = 30$ reveals the occurrence of an inter-individual mosaicism of Robertsonian origin in *Uranoscopus scaber* from the Gulf of Palermo. This mosaicism is clearly due to the fusion of one element of pair 10 with one element of pair 11, B- and C-type complements being the heterozygous and homozygous conditions for the fused elements respectively.

Hsu *et al.* (1975) proposed that such a fusion could produce a monocentric or dicentric bi-armed chromosome. However, the latter hypothesis must be discarded due to the absence, in the fused metacentrics, of a heterochromatin size corresponding to that derived from the fusion of one homologue of pair 10 with one of pair 11.

Based on the consideration that, in *U. scaber*, the Robertsonian fusion between two bi-armed chromosomes gives rise to a morphologically non-differentiated metacentric, it is clear that the short arms of the original chromosomes involved in the translocation have been lost. As abnormal specimens were not encountered in the population examined, we are inclined to accept the hypothesis of Bertollo *et al.* (1979), that the missing chromosomal portions possibly contain gene loci not responsible for functions of vital importance.

Forty-eight mono-armed chromosomes are considered to be the ancestral karyotype in fish (Ohno, 1974). This may suggest that the A-type is primitive in the population under study. Moreover, the presence of the fundamental numbers 56, 54 and 52, which are close to the ancestral number of 48, and the presence of a high number of large metacentric chromosomes in

the actual *U. scaber* specimens, seems to support the derivation of the A-type complement through numerous Robertsonian fusions occurring during the evolution of this species.

Another type of variation was observed in three specimens; however, because a comparison of the total number of cells analysed per specimen reveals that it was confined to only a few spreads, we have classified it as a low level intra-individual mosaicism. In particular, when the G-banding procedure was conducted on a single mosaic specimen with $2n=28$ and 29, it was observed that all three mutant cells encountered showed three elements with large secondary constrictions. This has allowed us to conclude that, in this case, trisomy involving pair 9 could be responsible for the intra-individual numerical variability. In contrast, as both mosaic specimens with $2n=30$ and 31 mutant cells contain an extra chromosome of the group of the medium-sized metacentrics (Fig. 1b), trisomy involving pairs 12 or 13 could have occurred.

Note that intra-individual mosaicism has been reported only in the genus *Salvelinus* (Davisson *et al.*, 1973; Hartley & Horne, 1984b) and in *Gobius fallax* (Thode *et al.*, 1988), while intra-populational mosaicisms in the form of Robertsonian translocations are widespread in teleostean fish. The latter were described in species of the same genus (Chen, 1971), in different populations of the same species (Black & Howell, 1978; Ojima & Kashiwagi, 1981) and in specimens of the same population (Ojima & Kashiwagi, 1981; Hartley & Horne, 1984a and b; Vitturi *et al.*, 1984; Thode *et al.*, 1985; Vitturi & Catalano, 1988; Vitturi, 1988).

Chromosomal variability involving secondary constrictions of pair 9 was also observed among different specimens. Inter-individual variability can be attributed to a varying amount of rDNA genes. This conclusion is supported by at least two arguments. The first is that secondary constrictions vary in length from one specimen to another but are uniform within the same individual; the second is that these areas are variable in length with all techniques employed, thus indicating that a change in the ribosomal genes has occurred.

Variability in the size of active NORs regularly occurs in teleostean fish and it is mainly explained as a functionally differentiated activity of RNA genes (Gold, 1984; Garcia *et al.*, 1987; Lopez *et al.*, 1989). On the other hand in some species of the order Gimnotiformes (Foresti *et al.*, 1981), in *Dicentrarchus labrax* and *D. punctatus* (Perciformes) (Vitturi *et al.*, 1990) and in *Bothus podas* (Pleuronectiformes) (Vitturi & Catalano, 1991), it has been suggested that NOR polymorphism could have a genetic rather than transcriptional basis.

Secondary constrictions of pair 9 stain like most of the homogeneously staining regions (HSRs) (Trent *et al.*, 1984), i.e. light after non-differentially staining and G-banding, moderately stained with C-banding and darkly stained with silver nitrate when composed of ribosomal DNA. It is widely held that HSRs are cytological manifestations of DNA sequence amplification and that they are characterized by displaying extreme fragility (Cowell, 1982). If this is true for the secondary constrictions of pair 9, wide heteromorphism of these areas would find a convincing explanation.

Acknowledgements

This research was supported by a 60 per cent MPI grant during 1988–1989.

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