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Localisation of NORs and counterstain-enhanced fluorescence studies in *Salmo gairdneri* and *Salmo trutta* (Pisces, Salmonidae)

B. Mayr [1], P. Rab [2] and M. Kalat [3]

[1] Institute for Animal Breeding and Genetics, Veterinary University, A-1030 Vienna, Austria
[2] Czechoslovak Academy of Sciences, Institute for Animal Physiology and Genetics, Department of Genetics, CS-277 21 Libechov, Czechoslovakia
[3] Ludwig Boltzmann Institute for Immuno- and Cytogenetic Research, Veterinary University, A-1030 Vienna, Austria

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Summary. The karyotypes of the rainbow trout (*Salmo gairdneri* R.) and the brown trout (*Salmo trutta* L.) were analyzed by means of silver staining and the chromomycin A_3/distamycin A/DAPI fluorescence banding technique. The nucleolus organizer regions (NORs) were localized at the secondary constrictions of chromosome no. 14 in *S. gairdneri* and of chromosome no. 10 in *S. trutta*. Additional silver positive dots were observed at or close to several centromeres in *S. gairdneri*. Brilliant chromomycin A_3 (CMA_3) fluorescence heterochromatin blocks were localized on both sides of the nucleolar constrictions in *S. gairdneri*. A polymorphic CMA_3 positive band was detected close to the NORs of *S. trutta*. No distamycin A/DAPI intense heterochromatin blocks were detected in the genomes of the two *Salmo* species investigated.

Key words: Nucleolus organizer – Chromomycin A_3 – Fluorescence – *Salmo gairdneri* – *Salmo trutta*

Introduction

Silver (Ag) staining of the nucleolus organizer regions (NORs) is one of the methods used for detecting the position of the gene complex for 18 S and 28 S rRNA in chromosomes (Goodpasture and Bloom 1975). Although considerable knowledge about the localisation of Ag NORs in the genomes of many vertebrates has accumulated during the last decade, there is a lack of silver-staining results in many fish families, e.g. the family Salmonidae. The application of C-banding or Q-banding techniques have led to the detection of heterochromatin markers in *S. gairdneri* (Thorgaard 1976; Ueda et al. 1984), *S. trutta* (Zenzes and Voicelescu 1975), *Salvelinus fontinalis* (Ueda and Ojima 1983) and *Salvelinus malma* and *Salvelinus namaycush* (Phillips and Zajicek 1982; Ueda and Ojima 1983). Results from sequential silver-staining-procedures and counterstain enhanced fluorescence methods have not been reported in Salmonidae so far.

In this study we investigated the chromosomes of the salmonids *S. gairdneri* and *S. trutta* by sequential silverstaining and counterstain enhanced fluorescence.

Material and methods

Ten *Salmo gairdneri* (5 males, 5 females) and 10 *S. trutta* (7 males, 3 females) were examined. These animals originated from Czechoslovakian and Austrian hatcheries. Standard procedures for chromosome preparation were used. Silver-NOR-staining was performed following the technique of Kodama et al. (1980). Subsequent to silver staining and photography the same metaphases were subjected to chromomycin A_3/Distamycin A/DAPI-(CDD) counterstaining (Schweizer 1981). By photographing selected cells with different filter combinations (e.g. Reichert filter blocks B_1 and U_1), both the chromomycin A_3 and the DA/DAPI staining could be documented. Conventional Giemsa staining was also performed. For chromosome nomenclature, see the Giemsa stained karyotypes in Figs. 3 and 4.

Results

In chromosomes of *S. gairdneri*, major silver NORs were detected on the secondary constrictions of the metacentric chromosome pair no. 14. Additionally, several chromosomes harboured prominent silver positive dots at or close to their centromeric regions (Fig. 1a). Striking examples are chromosomes no. 1, 2, 10, 15, 16, 22 and 23.

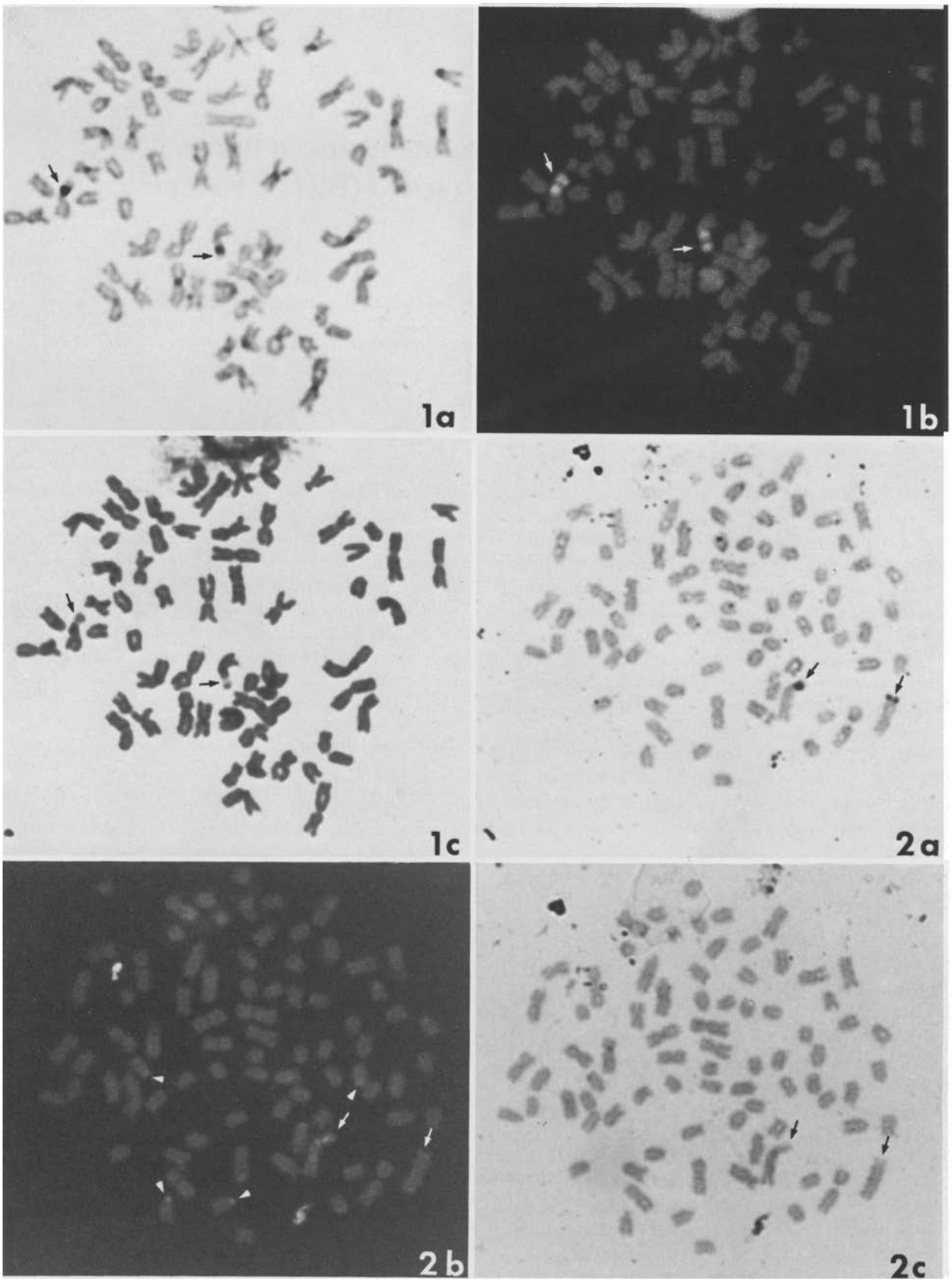
1a
1b
1c
2a
2b
2c

Fig. 3. CMA_3 (*upper lines*) and Giemsa (*lower lines*) karyotype of *S. gairdneri.* Note the brilliant CMA_3 blocks (*arrows*) flanking the NORs on chromosome 14

Chromomycin A_3 (CMA_3) produced a faint and a not very satisfactory differentiated fluorescent banding pattern along the metaphase chromosome arms. However, well-defined brilliant CMA_3-positive clusters flanking the nucleolus organizer regions were prominent. The NORs themselves appeared as a CMA_3-negative.

No prominent silver positive dots were found at the centromeres of *S. trutta* chromosomes. The Ag-NORs of *S. trutta* were localized at secondary constrictions of the short arms of the subtelocentric chromosome no. 10. The CMA_3 negative NORs in *S. trutta* were not flanked by brilliant CMA_3 positive clusters. However, a CMA_3 positive terminal band was localized close to the NOR constriction. This CMA_3 responsive band of a medium fluorescent intensity was highly polymorphic in size and thus makes its homing chromosome appear more subtelo- or acrocentric (Fig. 5). Prominent CMA_3

Figs. 1 and 2a–c. Sequential Ag/CDD/Giemsa staining of lymphocyte metaphases of *S. gairdneri* (**1**) and *S. trutta* (**2**). **a** Ag-NOR-staining. Two Ag-NORs (*arrows*) are localized on two chromosomes: no. 14 in *S. gairdneri* and no. 10 in *S. trutta.* Note the silver positive dots at, or close to, the centromeres of several chromosomes of *S. gairdneri;* **b** Chromomycin A_3 staining. Note the brilliant fluorescent clusters (*arrows*) flanking the NORs in *S. gairdneri.* In *S. trutta,* note the CMA_3 positive bands close to the NORs (*arrow*), on a subtelocentric (no. 11) and a telocentric (no. 23) chromosome pair (*arrowheads*); **c** Giemsa staining. Note the secondary constrictions in the NOR-regions (*arrowhead*)

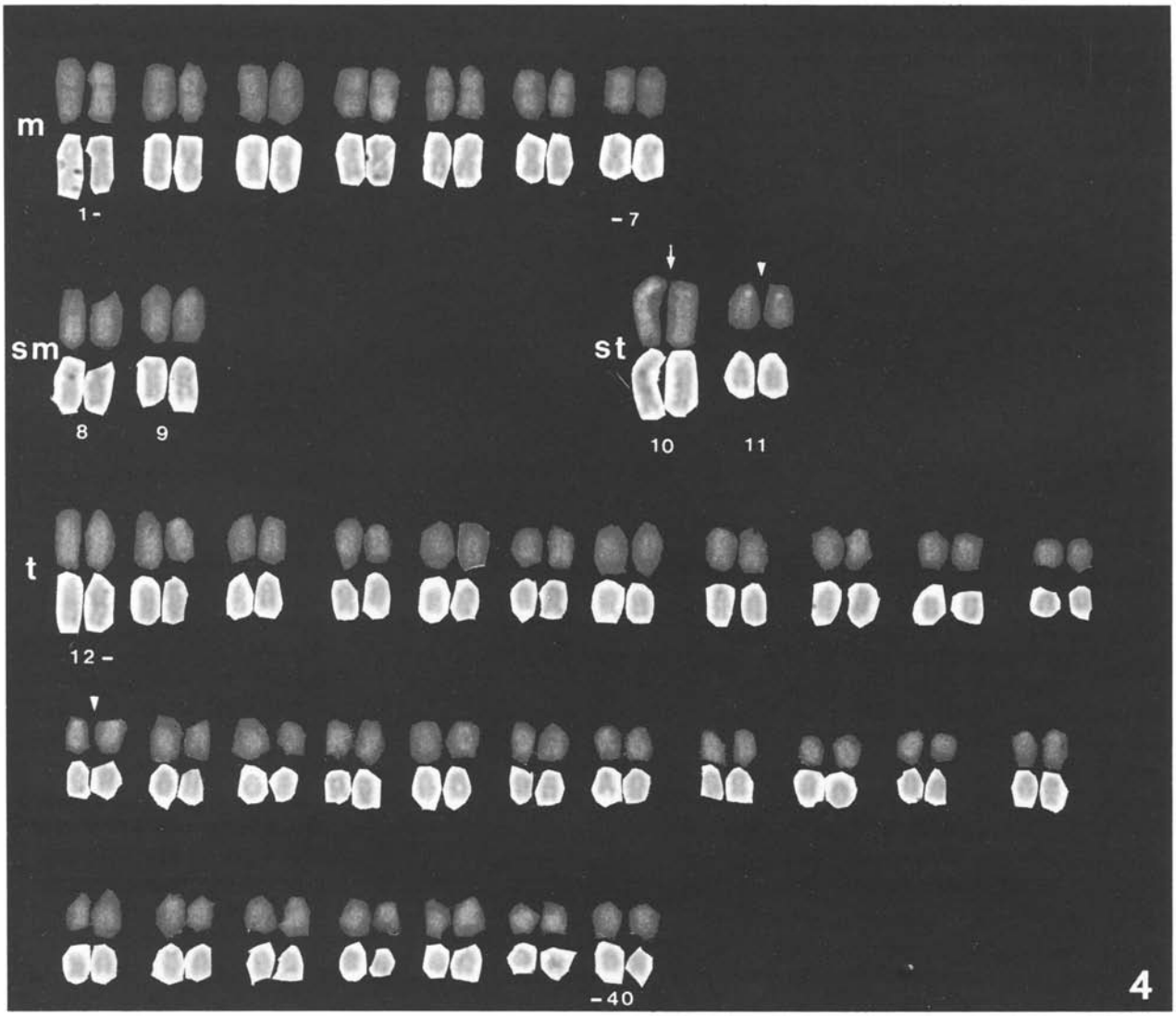

Fig. 4. CMA_3 (*upper lines*) and Giemsa (*lower lines*) karyotype of *S. trutta*. The *arrows* indicate a CMA-positive band close to the NORs; the *arrowheads* indicate centric CMA_3-positive bands on chromosome 11 and 23

positive clusters were detected on the centromeres of two chromosome pairs: 11 and 23 (Fig. 4). As in *S. gairdneri* the induced CMA_3 banding patterns along the chromosomes arms remained unsatisfactory in *S. trutta.*

Distamycin A/DAPI analyses of the metaphases did not selectively highlight certain heterochromatin regions either in *S. gairdneri* or *S. trutta;* the Distamycin A/DAPI patterns along the chromosomes were rather uniform.

Discussion

The application of silver staining to the chromosomes of *S. gairdneri* led to the selective staining of the NORs and of dots at or close to several centromeres. Silver nitrate may react with a number of nuclear structures in such cytological preparations as chromosomal NORs and interphase nucleoli, kinetochores or centromeric dots (Brown and Loughman 1980; Buys and Osinga 1982), in corelike structures of chromatids (Howell and Hsu 1979) and, in meiotic cells, the synaptonemal complexes (Dresser and Moses 1979). It is, therefore, not surprising that in our study the staining of the Ag-NORs was accompanied by silver staining of certain centromeres. Whether there is a similar basis and mechanism for the Ag-staining of the NORs and the centromeres cannot be answered conclusively at present and awaits further histochemical and biochemical analysis. Such investigations would also be in-

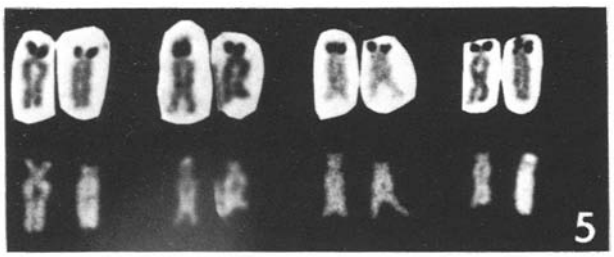

Fig. 5. NOR Chromosome pairs no. 10. of four individuals of *S. trutta*. Note the heteromorphic appearance of the NOR regions (*upper line* Ag-NOR staining, *lower line* CMA_3 staining)

dispensable in order to explain our observed pronounced differences in the silver staining behaviour of *S. gairdneri* and *S. trutta* centromeres. Only a few species of fish have been investigated by a sequential counterstain enhanced fluorescence procedure (CDD-technique) after silver NOR-staining (Mayr et al. 1985). In the perch, the NORs appeared as brilliantly CMA_3 fluorescent blocks. These results disagree with our present findings in *S. gairdneri* and *S. trutta*. In these two salmonidae, this intimate spatial interrelationship of silver NOR-positive and CMA_3 positive staining was not observed; instead, CMA_3 positive heterochromatic areas were either considerably separated from nucleolar constrictions, thus appearing as two brilliantly flanking fluorescent clusters (*S. gairdneri*) or as a neighbouring band of medium intensity (*S. trutta*). The antibiotic CMA_3 preferably forms stable complexes with GC-rich double-stranded DNA (Behr et al. 1969). Thus, it may be assumed that a high GC content in adjacent respective DNA-sequence is responsible for the chromomycin A_3 positive staining in the neighbourhood of the NORs. At present, the possible regulatory role of these repetitive sequences upon ribosomal gene activities cannot be excluded. The lack of DA/DAPI-positive heterochromatic clusters in both the fish species investigated is similar to results found in the perch (Mayr 1985). However, this staining procedure uncovers the presence of large amounts of a special AT-rich heterochromatin in the fish *Poecilia shenops* (Haaf and Schmid 1984).

References

Behr W, Honikel K, Hartmann G (1969) Interaction of the RNA polymerase inhibitor chromomycin with DNA. Eur J Biochem 9:82–92

Brown PA, Loughman WD (1980) Visible light observations on the kinetochore of the Indian muntjac, *Muntiacus muntjac*. Cytogenet Cell Genet 27:123–128

Buys CH, Osinga J (1982) A relation between GC- and N-band patterns as revealed by progressive oxidation of chromosomes and a note on the nature of N-band. Genetica 58:3–9

Dresser ME, Moses MJ (1979) Silver staining of synaptonemal complexes in the surface spreads for light and electron microscopy. Exp Cell Res 121:416–419

Goodpasture C, Bloom SE (1975) Visualisation of the nucleolus organizer regions in mammalian chromosomes using silver staining. Chromosoma 53:37–50

Haaf D, Schmid M (1984) An early stage of *ZW/ZZ* sex chromosome differentiation in *Poecilia sphenops* var. 'melanistica' (Poecilidae, Cyprinodontiformes). Chromosoma 89:37–41

Howell WM, Hsu TC (1979) Chromosome core structure revealed by silver staining. Chromosoma 73:61–66

Kodama Y, Yoshida MC, Sasaki M (1980) An improved silver staining technique for nucleolus organizer regions by using nylon cloth. Jpn J Hum Genet 25:229–233

Mayr B, Rab P, Kalat M (1985) Localisation of NORs and counterstain enhanced fluorescence in *Perca fluviatilis* (Pices, Percidae). Genetica 67:51–56

Phillips RB, Zajicek KD (1982) Q-band chromosomal polymorphisms in lake trout (*Salvelinus namaycush*). Genetics 101:227–234

Schweizer D (1981) Counterstain enhanced chromosome banding. Hum Genet 57:1–14

Thorgaard GH (1976) Robertsamian polymorphism and constitutive heterochromatin distribution in chromosomes of the rainbow trout (*Salmo gairdneri*). Cytogenet Cell Genet 17:174–184

Ueda T, Ojima Y, Sato R, Fukuda Y (1984) Triploid hybrids between female rainbow trout and male brook trout. Bull Jpn Soc Sci Fish 50:1331–1336

Ueda T, Ojima Y (1983) Karyotypes with C-banding patterns of two species in the genus *Salvelinus* of the family Salmonidae. Proc Jpn Acad, Ser B 59, 13:343–346

Zenzes MT, Voiculescu I (1975) C-banding patterns in *Salmo trutta*, a species of tetroploid origin. Genetica 45:531–536