# Restriction enzyme banding in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*)

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**Summary**

Fixed metaphase chromosomes of brown trout and Atlantic salmon were digested with various restriction enzymes and stained with Giemsa. C band-like patterns were produced in both species by *Alu* I, *Dde* I, *Hae* III and *Mbo* I. *Alu* I revealed extra chromosome bands in brown trout which allowed identification of additional chromosome pairs, while the other three enzymes produced patterns identical to C banding. In the Atlantic salmon *Dde* I revealed telomeric bands at all telomeres in addition to the conventional C bands and all four enzymes had differential effects on the nucleolar organizer-associated heterochromatin. The relevance of these findings to chromosome identification and constitutive heterochromatin organization in salmonid fishes is discussed.

## 1. Introduction

Restriction enzymes, which cleave DNA at specific recognition sequences, produce banding patterns in fixed metaphase chromosomes by cleavage and loss of DNA (Babu, 1988). The extent to which DNA is extracted from a chromosomal region depends on the frequency or accessibility of the recognition sequence in that region. Regions either resistant to digestion or containing few accessible sites stain darkly with Giemsa following digestion and many of the enzymes used generate C band-like patterns. In mammals, amphibians and insects restriction enzyme digestion of chromosomes has provided evidence for subclasses of highly repeated DNA in the constitutive heterochromatin both within species and between closely related species (Miller *et al.* 1983; Babu & Verma, 1986; Ferrucci *et al.* 1987; Gosalvez *et al.* 1987; Schmid & de Almeida, 1988).

In the two fishes studied to date, modified C-banding patterns have been found following treatment of chromosomes with restriction enzymes. In the anguilliform fish, *Muraena helena*, the existence of different classes of highly repetitive DNA in centromeres and telomeres was demonstrated following digestion with *Hae* III, *Dde* I and *Mbo* I (Cau *et al.* 1988). In the rainbow trout (*Oncorhynchus mykiss*) more distinct C-banding patterns, with more telomeric bands, were obtained following treatment with *Alu* I, *Hae* III, *Hin* fI, *Mbo* I and *Pvu* II than with conventional C banding (Lloyd & Thorgaard, 1988).

The Atlantic salmon (*Salmo salar*) and the brown trout (*Salmo trutta*) are salmonid fish which are closely related enough to hybridize naturally producing viable offspring (Verspoor, 1988; Garcia de Leaniz & Verspoor, 1989) but have different chromosome numbers and different amounts of constitutive heterochromatin as revealed by C banding (Hartley & Horne, 1984*a*). The Atlantic salmon is unusual among salmonids in having a diploid chromosome number ($2n$) of 58 and a chromosome arm number (NF) of 74. The brown trout has a more typical karyotype of $2n = 80$ with an NF of 100 (Hartley, 1987). C banding reveals relatively little heterochromatin in the brown trout other than that found at the centromeres and associated with the nucleolar organizer regions (NORs), although there are four submetacentric chromosomes with C-banded short arms (Hartley & Horne, 1984*a*). In the Atlantic salmon, in addition to centromeric bands, many of the metacentric chromosomes have telomeric C bands, the large acrocentric chromosomes possess interstitial bands and a large polymorphic block of heterochromatin is associated with the NORs (Hartley & Horne, 1984*b*; Hartley, 1988). In this study restriction enzymes have been used to further investigate the chromosomes of these two species.

## 2. Materials and methods

Chromosome preparations were made from lymphocyte cultures of 10 brown trout and 10 Atlantic salmon as described previously (Hartley & Horne, 1983, 1985). The restriction enzymes used were *Alu* I,



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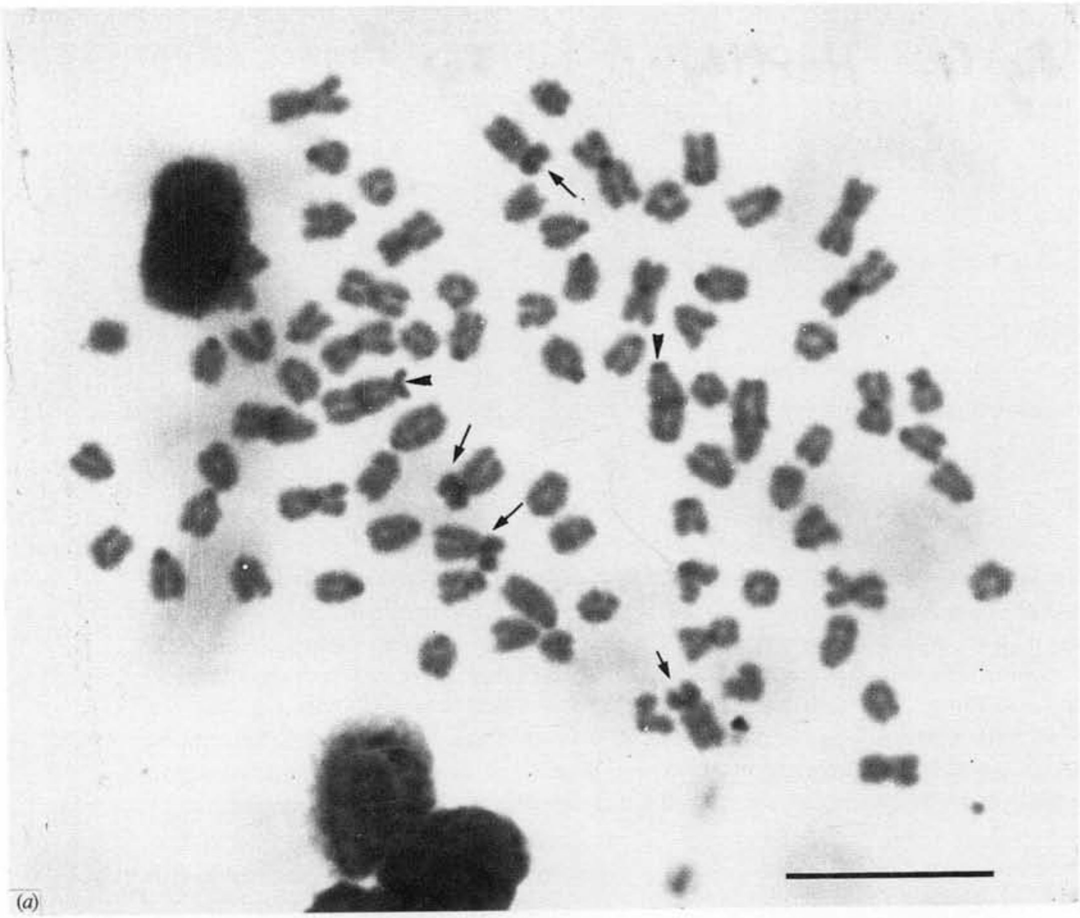

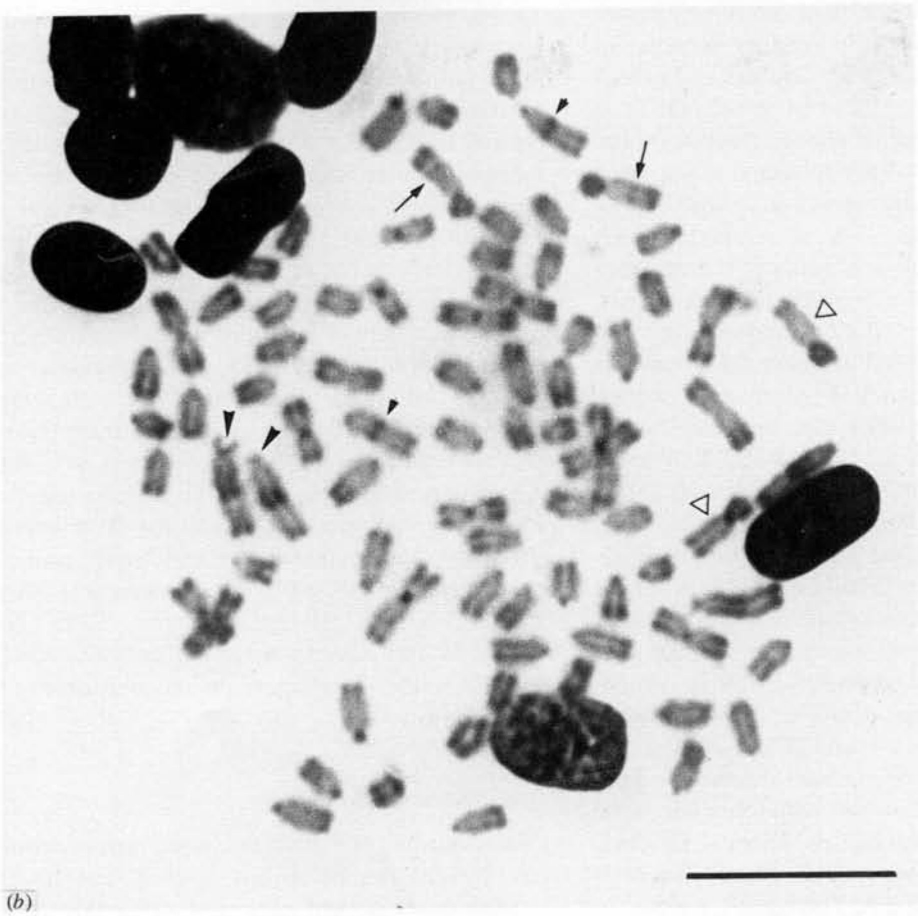

Fig. 1. Metaphases of brown trout following (*a*) C banding and (*b*) digestion with *Alu* I. In (*a*) the NOR-bearing chromosomes are indicated by large arrowheads and the four subtelocentric chromosomes with C-banded short arms are indicated by arrows. In (*b*) large arrowheads indicate the NOR bearing chromosomes, the subterminal band in one pair of subtelocentrics is indicated by arrows, the other subtelocentric pair are indicated by open triangles and small arrowheads indicate the third acrocentric pair with an interstitial band. Bar, 10 $\mu$m.

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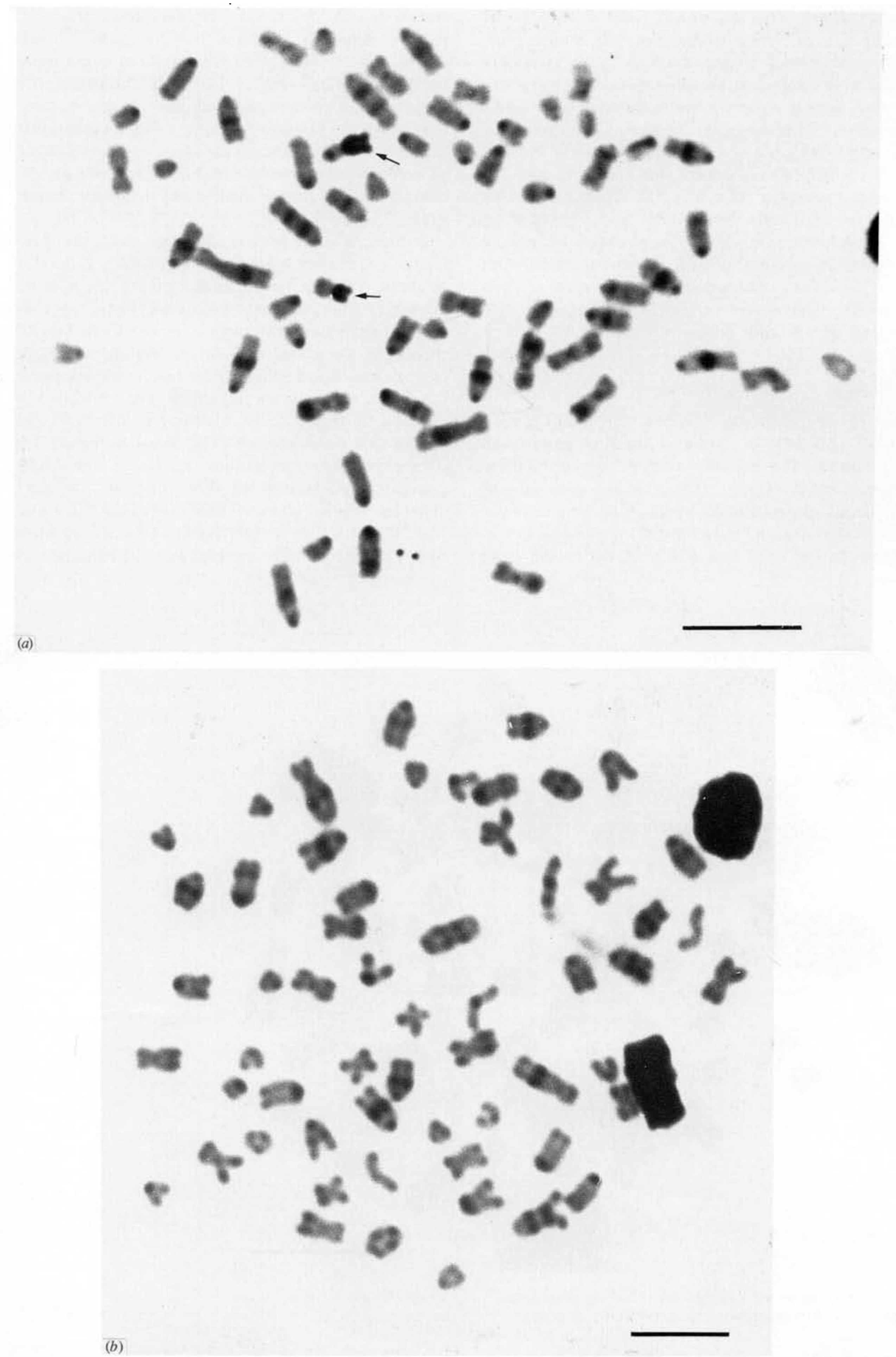

Fig. 2. Metaphases of Atlantic salmon following (*a*) C banding and (*b*) digestion with *Alu* I. The large polymorphic blocks of heterochromatin associated with the NORs are arrowed in (*a*), but are absent in (*b*). Bar, 10 $\mu$m.

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*Ava* II, *Dde* I, *Hae* III, *Hin* fI, *Mbo* I and *Pvu* II (Pharmacia or Gibco-BRL). For chromosome digestion, 15 units of enzyme in 100 $\mu$l of the appropriate buffer were applied to the chromosome preparation, covered with a coverslip and incubated in a moist chamber at 37 °C overnight. Following incubation the coverslip was removed by washing with distilled water, which also stopped the reaction, and the chromosomes were stained in 4% Giemsa in pH 6·8 buffer for 10–20 min. Some slides were C banded by the method of Sumner (1972). Between 10 and 20 metaphases were examined for each treatment from each individual. Slides were viewed with a Zeiss Universal microscope on bright field and photographed with Kodak Technical Pan film.

## 3. Results

Four of the restriction enzymes used (*Alu* I, *Dde* I, *Hae* III and *Mbo* I) produced banding patterns in both species. The patterns were all similar to those obtained by C banding (Figs. 1*a*, 2*a*) although for *Hae* III and *Mbo* I the difference in staining intensity between banded and non-banded regions was not as distinct as for *Alu* I and *Dde* I. In the brown trout digestion with *Dde* I, *Hae* III and *Mbo* I produced patterns identical to the C-banding pattern while digestion with *Alu* I (Fig. 1*b*) results in many more bands becoming visible. The four submetacentric chromosomes with C-banded short arms become resolvable into two pairs as one pair has an additional band in the long arm; a third pair of acrocentric chromosomes become identifiable because of a prominent interstitial band; and many telomeric bands become visible.

In the Atlantic salmon digestion with the four restriction enzymes produces essentially C-banded patterns. The main difference between the C-band pattern and that obtained with *Alu* I (Fig. 2*b*) is that the chromosomes possessing large blocks of heterochromatin associated with the NORs are no longer identifiable. *Dde* I (Fig. 3) reveals extra telomeric bands so that all the telomeres are banded and appears to digest some centromeric bands in the metacentric chromosomes. The major difference between the enzymes is their effect on the NOR associated heterochromatin (Fig. 4*a*). Following *Alu* I digestion it is no longer visible, following *Dde* I and *Hae* III it is visible as pale regions (Fig. 4*b*, *c*) while *Mbo* I leaves it unaffected (Fig. 4*d*). No banding was

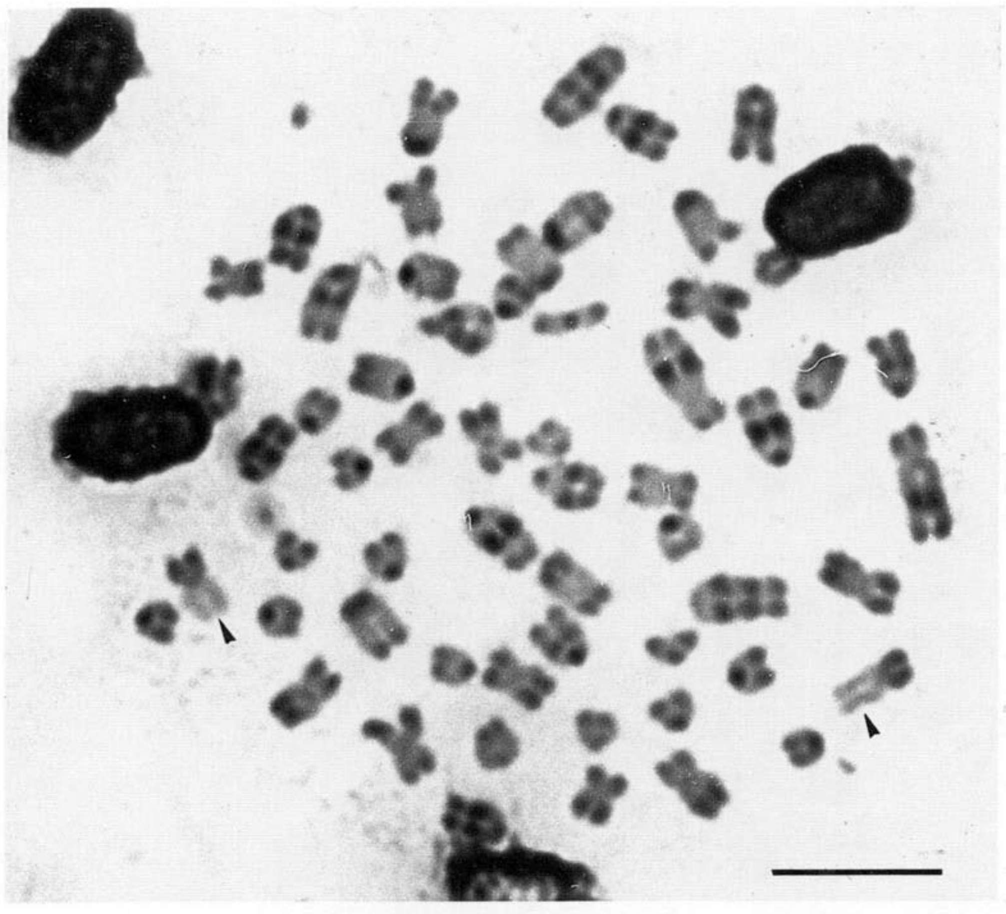

Fig. 3. Atlantic salmon metaphase following digestion with *Dde* I. Arrowheads indicate the NOR-associated heterochromatin. Bar, 10 $\mu$m.

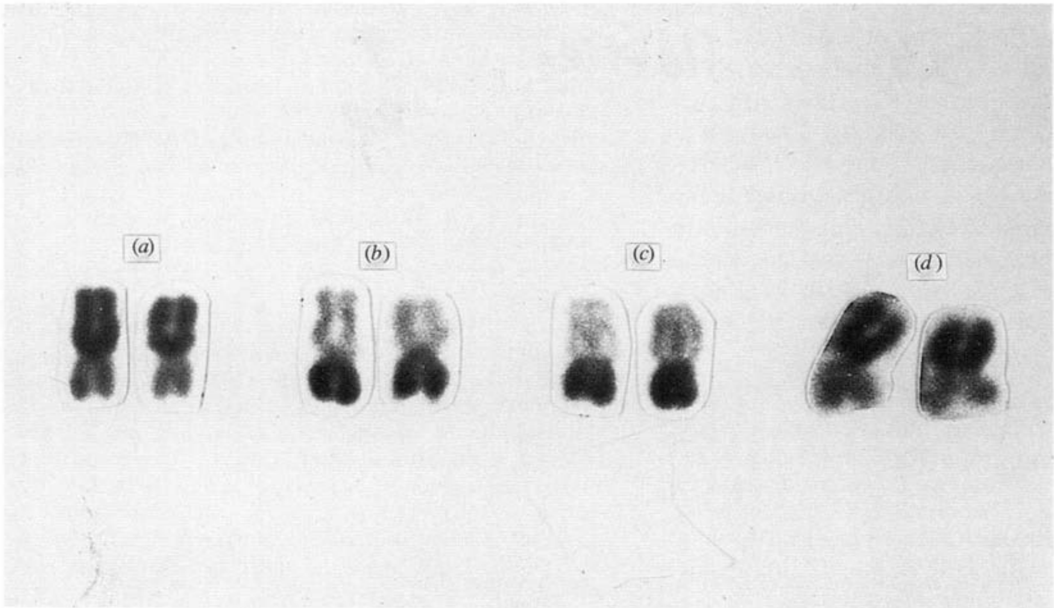


Fig. 4. The NOR-bearing chromosomes of Atlantic salmon following (*a*) C banding, (*b*) *Dde* I digestion, (*c*) *Hae* III digestion and (*d*) digestion with *Mbo* I.

obtained in either species when chromosomes were incubated in buffer alone or with *Ava* II, *Hin* fI and *Pvu* II.

## 4. Discussion

Restriction enzyme banding may serve a dual purpose in the study of fish chromosomes: to provide additional information for chromosome identification and to study the repetitive components of the genome. Chromosome identification in fishes, particularly for those species such as the salmonids with large numbers of small chromosomes, has been hampered by the failure to obtain detailed linear banding patterns such as those found in higher vertebrates. This failure is thought to be due to the lack of genome compartmentalization, i.e. the scarcity of GC-rich isochores in the DNA (Medrano *et al.* 1988).

In those species with little conventional C banding other than centromeric bands, such as the brown trout and the rainbow trout, restriction enzyme digestion is a useful tool for providing extra chromosome bands. Thus, in the *Alu* I digested brown trout chromosomes extra bands have permitted the identification of more chromosome pairs than with C banding and *Alu* I banding in rainbow trout permitted identification of several homologous pairs (Lloyd & Thorgaard, 1988). The Atlantic salmon possesses telomeric and interstitial C bands in the metacentric and large acrocentric chromosomes respectively. No extra bands are revealed by *Alu* I and although *Dde* I reveals telomeric bands in all the acrocentric chromosomes, this does not allow positive identification of any additional homologous pairs.

In general the constitutive heterochromatin which may be visualized by C banding contains the bulk of the highly repeated DNA in a genome, the sequence composition of which may vary within and between species (John, 1988). Restriction enzyme banding has revealed heterogeneity of heterochromatin within a species when chromosomes are digested with a range of restriction enzymes (e.g. Miller *et al.* 1983; Gosalvez *et al.* 1987; Marchi & Mezzanotte, 1988) and between related species when chromosomes are digested with the same enzyme (e.g. Ferrucci *et al.* 1987; Schmid & de Almeida, 1988). Similar results are obtained when fish chromosomes are digested with restriction enzymes.

Thus in *Muraena helena* differences between the telomeric and centromeric heterochromatin are found (Cau *et al.* 1988). In this study *Dde* I digestion of Atlantic salmon chromosomes suggests that there may be heterogeneity of the centromeric heterochromatin. Differences between species are revealed when salmonid chromosomes are digested with *Hin* fI and *Pvu* II. Banding patterns similar to C banding were obtained in rainbow trout (Lloyd & Thorgaard, 1988) but not in Atlantic salmon, brown trout, Arctic charr or brook trout (this study; Hartley, unpublished observation).

Digestion with restriction enzymes also allows study of the base composition of particular chromosomal areas. The NOR associated heterochromatin in Atlantic salmon and brown trout fluoresces brightly with the GC-specific fluorochrome chromomycin A3 (Phillips & Hartley, 1988). While it was difficult to assess the effect of restriction enzymes on the NOR associated heterochromatin of brown trout due to its small size in the individuals examined, there were quite dramatic effects on that of the Atlantic salmon. The most striking was the complete elimination of staining with *Alu* I whose recognition sequence is

AG!CT. *Hae* III (GG!CC) and *Dde* I (C!TNAG) reduced the staining of the region but *Mbo* I (!GATC) left it unaffected. These findings suggest that the NOR heterochromatin is poor in *Mbo* I sequences, contains moderate amounts of *Hae* III and *Dde* I sequences and is rich in *Alu* I sequences. The NOR heterochromatin of *Muraena helena* was also found to be poor in *Mbo* I sequences while containing moderate and large amounts of *Hae* III and *Dde* I sequences respectively (Cau *et al.* 1988). Whether this is a general feature of NOR associated heterochromatin remains to be investigated.

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