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Chromosome Banding Studies by Replication and Restriction Enzyme Treatment in Vendace (*Coregonus albula*) (Salmonidae, Salmoniformes)*

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Chromosome banding studies were performed in vendace, *Coregonus albula*. Original data on distribution of early and late replication regions, restriction sites (*Alu*I, *Dde*I, *Hinf*I and *Hae*III) on chromosomes in this coregonid fish have been used to analyse karyotype heterochromatin differentiation. Heterochromatic bands (C-positive and not digested by restriction enzymes) have been identified as late replicating regions. Extra bands produced by the applied methods have permitted the identification of several homologous pairs. The centromeres were differentially digested by the restriction enzymes. The studied population seems to be homogenic regarding karyotype characteristics.

Key words: Cytogenetics, fish, karyotype, restriction enzyme banding, replication banding.

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Coregonid fishes (Salmoniformes, Salmonidae) are broadly distributed in Holarctic waters. The vendace (*Coregonus albula*) is a polymorphic species of circumpolar distribution composed of several intraspecific forms (VUORINEN *et al*. 1981; BODALY *et al*. 1991).

The common ancestor of salmonid fishes underwent an autotetraploidization event, followed by rediploidization, i.e. the preferential formation of two separate bivalents instead of one quadrivalent following genome duplication (ALLENDORF & THORGAARD 1984). Different Salmonid groups have undergone different degrees of diploidization, and the number of chromosomes has been reduced by Robertsonian fusions (HARTLEY 1987). Mechanisms such as chromosome inversion and fusion have played a major role in the karyotype evolution of salmonid fishes after autotetraploidization (ALLENDORF & THORGAARD 1984).

The karyotype of vendace consists of 80 chromosomes with NF=96 (NYGREN *et al*. 1971; JANKUN *et al*. 1991, 1995). Detailed characteristics of the location and expression of nucleolar organizer regions (NORs) have been put forward by JANKUN *et al.* (2000, 2001), however, the organization of heterochromatin is almost unknown. Chromosomes of higher eucaryotes are composed of a number of repetitive DNA regions that may have played an important role in determining chromosomal shape and may have affected the divergence of chromosomes by mutation.

The purpose of this study was to analyze the vendace chromosome set with an emphasis on the distribution of early and late replication bands and heterochromatin differentiation with restriction enzymes.

Material and Methods

Twenty seven specimens of *Coregonus albula* (vendace) were obtained from a natural population (Wulpinskie Lake, Olsztyn district, Poland) and were subjected to cytogenetic investigation.

Chromosomal preparations were obtained from cell suspensions of the cephalic portion of the kidney of each fish according to JANKUN *et al*. (1991) and from cell cultures of the cephalic kidney cells

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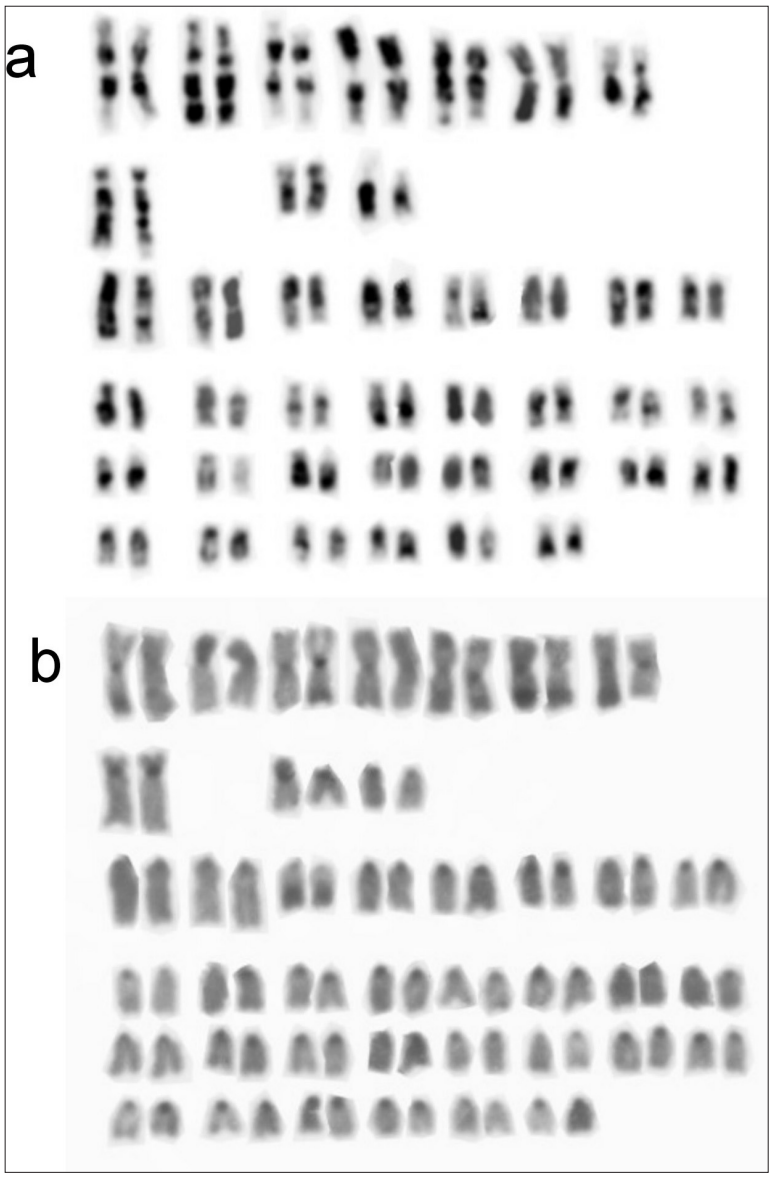

Fig. 1. Karyotype of vendace (*Coregonus albula*). Homologues were identified by (a) replication bands, (b) C-banding.

according to MARTINEZ *et al.* (1991). In total 450 metaphase plates were analysed.

Replication banding was performed according to JANKUN *et al.* (1998). *In vivo* BrdU incubation equalled 12 h.

Restriction endonucleases suspended in an appropriate buffer were added to one drop of an air-dried cell suspension and covered with a 24 x 32 mm coverslip. The concentration of each enzyme (*Alu* I, *Dde* I, *Hin* fI, *Hae* III) varied from 0.3 to 0.5 U/μl, depending on its activity. Slides were incubated in a moist chamber at 37^{o}C for 2-2.5 h, washed with distilled water, and stained with 5% Giemsa for 5 min.

The constitutive heterochromatin blocks were visualised by a C-banding technique as described by SUMNER (1972).

Results

Replication banding

Replication bands were visualised as light and dark areas on chromosomes in *Coregonus albula*.

Specific banding patterns in vendace were located on all biarmed and some uniarmed chromosomes (Fig. 1a). The late replicated chromatin blocks were observed on both arms of chromosome pairs 1 and 3 and on six small pairs of acrocentric chromosomes. Interstitial blocks were also present. They were located on two pairs of biarmed (number 2 and 8) and five pairs of uniarmed chromosomes. Replication banding patterns enabled the identification of homologous chromosome pairs (Fig. 1a).

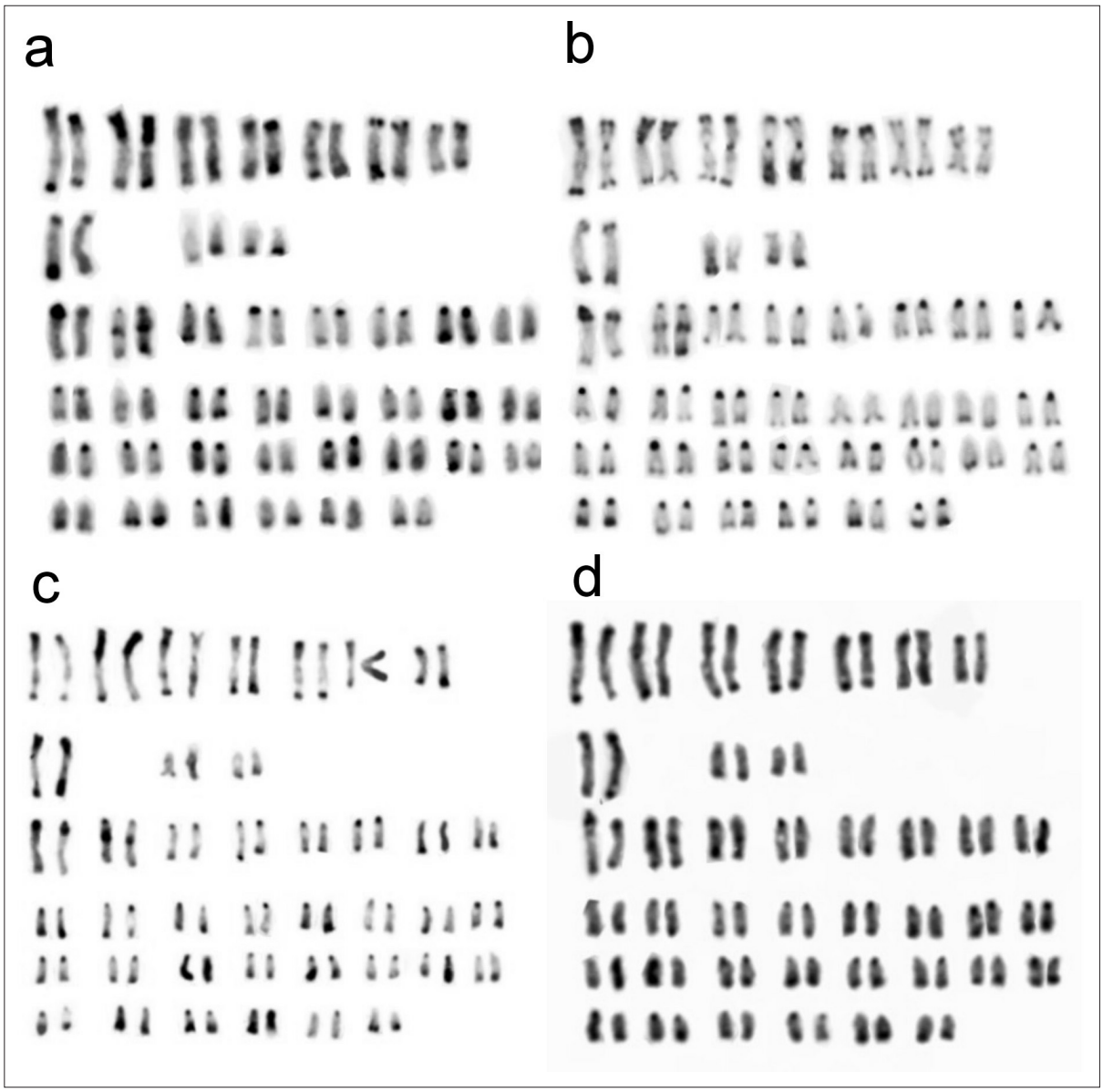

Fig. 2. Karyotype of vendace (*Coregonus albula*), chromosomes digested with (a) *Alu* I, (b) *Dde* I, (c) *Hae* III and (d) *Hin* fI.

C-banding

C-banding revealed the distribution of constitutive heterochromatin and showed centromere-connected heterochromatin in almost all chromosome pairs plus heterochromatic blocks on meta-submetacentric pairs (Fig. 1b).

Restriction enzymes

Four of the restriction enzymes used in the present study (*Alu* I, *Dde* I, *Hae* III, *Hin* fI) (Fig. 2) produced a specific and reproducible banding pattern in the vendace karyotype. For each enzyme representative metaphases were karyotyped for different individuals of *Coregonus albula* from both sexes. The patterns were all similar to those obtained by C-banding, although for *Hin* fI the differences in staining intensity between banded and non-banded regions were not as distinct as for *Alu* I and *Dde* I. Taking into account the chromosome size, the position of the centromere and the banding pattern induced by each enzyme used, the identification of several homologue pairs was possible.

Centromeric staining after *Alu* I and *Hae* III (Fig. 2a,c) digestion is absent in most of the biarmed chromosomes (except for three metacentric pairs), whereas in most of the uniarmed chromosomes centromeric staining is very clear (except for the tenth and seventh acrocentric pairs for *Alu* I and *Hae* III digestion, respectively). *Dde* I digested only centromeres of two biarmed chromosome pairs (number 5 and 8) and seven acrocentrics (Fig. 2b), whereas *Hin* fI only one metacentric and nine acrocentric chromosome pairs (Fig. 2d).

All endonucleases used in the study generated distinct telomeric bands (Fig. 2). Some interstitial

bands which were not detected by C-banding were produced by all restriction enzyme used.

Chromosome pairs one and three had a clear interstitial band on the long arm after digestion. All enzymes produced dark bands at the pericentromeric region of chromosome 11 (a large acrocentric) and an interstitial band on chromosome 12 (another large acrocentric). These were not detectable by C-banding.

A previous study concerning the vendace population from lake Ukiel (Olsztyn district, Poland, 20 km from Wulpinskie Lake) showed C-band positive heterochromatin length heteromorphism (JANKUN *et al*. 1995). In the present study these chromosome pairs (nos 2 and 5) (Fig. 1) can be classified respectively as type LL (metacentric with short arm completely heterochromatic) and SS (submetacentric with one band at distal part of the long arm) according to JANKUN *et al*. (1995). No chromosome heteromorphism nor differences between sexes was found.

Discussion

Chromosome identification in fishes, particularly for those species such as the salmonids with large numbers of small chromosomes, has been hampered by a 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 (MEDRANO *et al*. 1988).

Replication and restriction enzyme banding may serve a dual purpose in the study of fish chromosomes: to provide additional information for chromosome identification and to study repetitive components of the genome. It has been proposed that replication banding patterns may be one of the most sensitive methods available for chromosome characterization in salmonids (PENDAS *et al*. 1993; AMARO *et al*. 1996; JANKUN *et al*. 1998). Homologous chromosomes have in general the same replication pattern, presumably because they share the same regional and temporal organization of replicon clusters (HOLMQUIST *et al*. 1982). Slight variation in both size and position of bands occurred. These differences have been interpreted in terms of a certain asynchrony between homologous replicons (SCHEMPP 1980), differences in the condensation state of homologous chromosomes or technical devices (DROUIN *et al*. 1988).

In this study, it has been demonstrated that BrdU incorporation to cells *in vivo* and subsequent FPG staining can be used to obtain a reproducible replication banding pattern in the chromosomes of vendace. The replication patterns obtained have permitted the identification of all bi-armed chromosome pairs, and some uni-armed ones.

In general, C-positive heterochromatin was late replicating, similarly as was reported for rainbow trout (DELANY & BLOOM 1984), Atlantic salmon (PENDAS *et al*. 1993; ABUIN *et al*. 1994) and European whitefish (JANKUN *et al*. 1998). However, not all very late replicating regions in the chromosomes are also C-band positive (Fig. 1). A similar observation was made by ABUIN *et al*. (1994) in *Salmo salar* and by SCHEMPP and SCHMID (1981) in Amphibia. These late replicating and C-band negative regions could still be heterochromatic since constitutive heterochromatin is known to consist of very heterogenous materials (BABU & VERMA 1987).

In those species with little conventional C-banding other than centromeric bands, such as vendace, restriction enzyme digestion is a useful tool for providing extra chromosome bands. Thus in the *Alu* I, *Dde* I, *Hae* III and *Hin* fI digested vendace chromosomes, extra bands have permitted the identification of several homologous pairs (Fig. 1). In salmonid fishes, several families of repetitive DNAs have been found which map to different centromeres (REED & PHILLIPS 1995; REED *et al*. 1997). Restriction enzyme banding has revealed heterogeneity of the centromeric heterochromatin within a species when chromosomes are digested with a range of restriction enzymes (HARTLEY 1991; LOZANO *et al*. 1991). Similar results have been obtained in vendace chromosomes.

In this study the centromeres showed a variable response to digestion with restriction enzymes. While most were digested with *Alu* I, the majority remained undigested with *Dde* I, and some were digested with *Hae* III and *Hin* fI.

A high degree of heterochromatinization of chromosome regions involved in the heteromorphism may influence chromosome rearrangements such as amplifications/deletions or translocations (SUMNER 1998). The chromosome arm size heteromorphism observed previously in a geographically close vendace population (JANKUN *et al*. 1995) has not been observed in fish from Wulpinskie Lake. The studied population seems to be homogenic regarding karyotype characteristics.

The interstitial banding pattern revealed by treatment with restriction enzymes and replication banding suggests some kind of chromatin differentiation along the chromosome arms in *Coregonus albula*. A high level of polymorphism concerning the number and location of NORs (JANKUN *et al*. 2001) as well as C-positive heterochromatin (JANKUN *et al*. 1995) shows that chromosome rearrangements and heterochromatinization occur in the *Coregonus albula* karyotype.

This study has shown that replication and restriction enzyme banding enable the detection of different types of heterochromatin in particular chromosomal areas and provide a better karyotypic definition of fish chromosomes.

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