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# Chromosome Study of Peled (*Coregonus peled*, Salmoniformes)*

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Chromosomes of *Coregonus peled* were examined by Giemsa, $CMA_3$, Ag-NOR and C-banding. The karyotype of peled had a diploid number 2n=76, arm number NF=96 and consisted of twenty meta-submeta chromosomes and 56 subtelo-acrocentric chromosomes. C-positive blocks of heterochromatin were observed on the telomeric regions of meta- and submetacentric chromosomes. Pairs no. 1 and 11 had short arms, completely heterochromatic. The NOR was observed at one acrocentric pair, no. 11. Arm length polymorphism was observed on the NOR-bearing pair.

Key words: Chromosome banding, cytogenetics, peled, NOR.

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The structure of the ichthyofauna of freshwater zoosystems in Poland has changed during the last decades. One of the causes of these changes is the introduction of new species, a method that became popular in fisheries. Peled, *Coregonus peled* was brought to Poland in 1966 and was next introduced into selected waters. Previously, the species never occurred in Polish inland waters. Peled is a coregonid which is naturally distributed in northern Asia, in the region between the Mezen (in the west) and Kolyma (in the east) rivers. In Poland there are two native coregonid species (whitefish, *Coregonus lavaretus* and vendace, *Coregonus albula*). Hybridization in numerous populations of coregonid fishes in European lakes has been documented (VUORINEN 1988; BODALY *et al*. 1991; LUCZYNSKI *et al*. 1999).

The karyotype of peled has been described in the literature (VIKTOROVSKIJ 1964; NYGREN *et al*. 1971; KAYDANOVA 1978, 1986; ANDRYASHEVA *et al*. 1982; JUNTUNEN 1987), but only basic features like chromosome number and chromosome arm number (NF) have been studied.

The aim of the present study was to describe the karyotype of peled, in order to compare chromosomes of coregonid species which exist in Polish waters, and find chromosomal markers.

## Material and Methods

This study was based on two peled groups: specimens aged 2+ from stock of the Taivalkoski Game and Fisheries Research Station (Finland) and specimens from the same stock at age 0+, which were shipped to Olsztyn as eyed stage embryos and then incubated, hatched and grown to a size of 7-8 cm. 26 fish were examined, chromosomes were prepared according to JANKUN *et al*. (1995a). The analysis of the number and morphology of chromosomes was based on conventional Giemsa staining. Nucleolar Organiser Regions (NORs) were analysed using fluorescent dye chromomycin $A_3$ ($CMA_3$) (SOLA *et al*. 1992) and Ag-NOR staining (HOWELL & BLACK 1980 with modification). Metaphases were investigated also by C-banding methods (HAAF & SCHMID 1984).

All specimens examined cytogenetically were frozen and stored at -20°C before electrophoretic analysis. Genotypes of individual fish were determined by horizontal starch gel electrophoresis of

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muscle samples as described by VUORINEN (1988). Two enzymes coded by three diagnostic loci: $NADP^+$ – dependent malic enzyme (1.1.1.40, sMEP) and superoxide dismutase (1.15.1.1, sSOD) (nomenclature according to SHAKLEE *et al*. 1989) were used.

## Results and Discussion

All examined fish were positively identified electrophoretically as *Coregonus peled.*

The distribution of diploid chromosome numbers of each peled specimen ranged from 74 to 76. The modal chromosome number and the chromosome arm number were 2n=76 and NF=96 in most specimens. The karyotype consisted of ten biarmed chromosome pairs and 28 uniarmed pairs (Fig. 1). In the case of five specimens, NF equaled 97 because of arm length polymorphism in the NOR-bearing chromosome pair 11. After silver and chromomycin-$A_3$ staining, one pair of NORs was found in the karyotype of the peled (Fig. 3). Chromomycin-$A_3$ staining showed GC rich heterochromatic blocks located in the entire short arm of the small subtelocentric pair in 21 specimens, whereas five specimens showed size polymorphism in this pair (Fig. 3d). Two forms were observed in the NOR-bearing locus: large with a double amount of NOR cistrons, and connected heterochromatin called L and S with a single amount of NOR cistrons and connected heterochromatin. Two cytotypes were observed in the studied stock: homozygotes SS and hetrozygotes LS (one homologue was submetacentric, whereas the other subtelocentric) (Fig. 3a, 3b).

The distribution of C-positive heterochromatin in the peled karyotype is presented in Figure 2. C-banding showed that the short (p) arm of the first metacentric chromosome pair and NOR-bearing pair were completely heterochromatic (Fig. 2).

There is only limited data about the peled karyotype (for a review see KAYDANOWA 1989). All examinations which have been done up to now focused on basic karyotype parameters like diploid chromosome number (2n) and chromosome arm number (NF).

The karyotype of peled analysed in the present work (2n=76, NF=96), was similar to that observed by JUNTUNEN (1987) and KAYDANOVA (1989). The difference in diploid chromosome number could be due to Robertsonian polymorphism. KAYDANOVA (1989) presents karyotypes 2n=74, 75 and 76. Two of them (2n=74 and 75) have 11 pairs of biarmed chromosomes (excluding the NOR-bearing ones), whereas the karyotype of 2n=76 consists of 10 biarmed pairs (excluding the NOR-bearing ones). Robertsonian polymorphism is a common phenomenon observed in salmonids (for a review see PHILLIPS & RAB 2001) but it has not been found in Polish coregonids.

C-banding on peled chromosomes was done only by JUNTUNEN (1987). The latter author also found a completely heterochromatic "p" arm of pair no. 1, and the same distribution of heterochromatin on pair no. 11, as observed in the present study (Fig. 2).

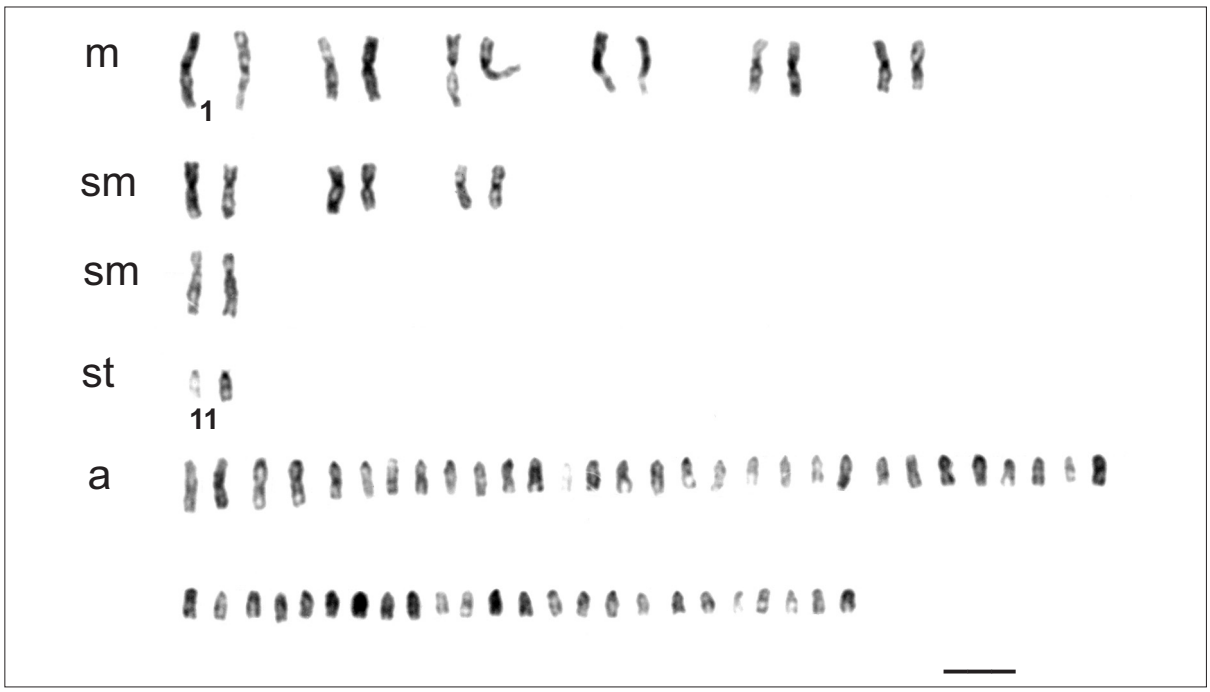


Fig. 1. Metaphase chromosomes of peled (*Coregonus peled*) 2n=76 and NF=96 stained with Giemsa (m – metacentric chromosomes, sm – submetacentric chromosomes, st – subtelocentric chromosomes, a – acrocentric chromosomes). Bar = 10 $\mu$m.

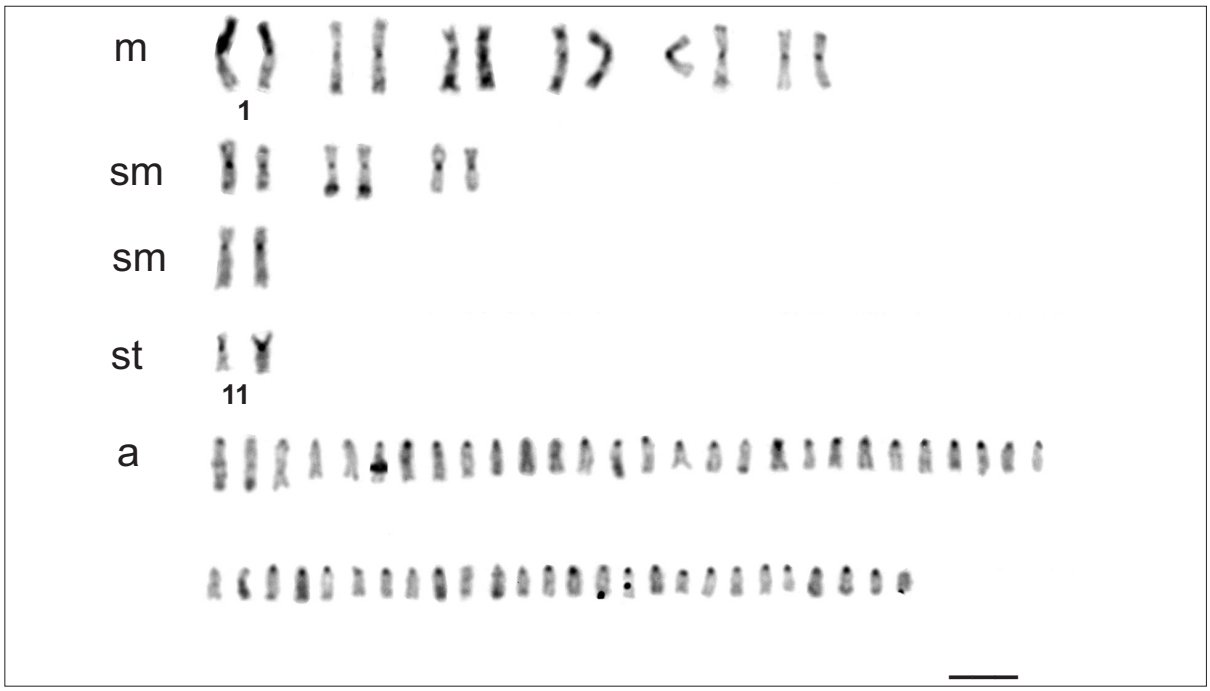


Fig. 2. Metaphase chromosomes of *Coregonus peled* after C-banding. Pairs no. 1 and 11 with heterochromatic short arms. Bar = 10 $\mu$m.

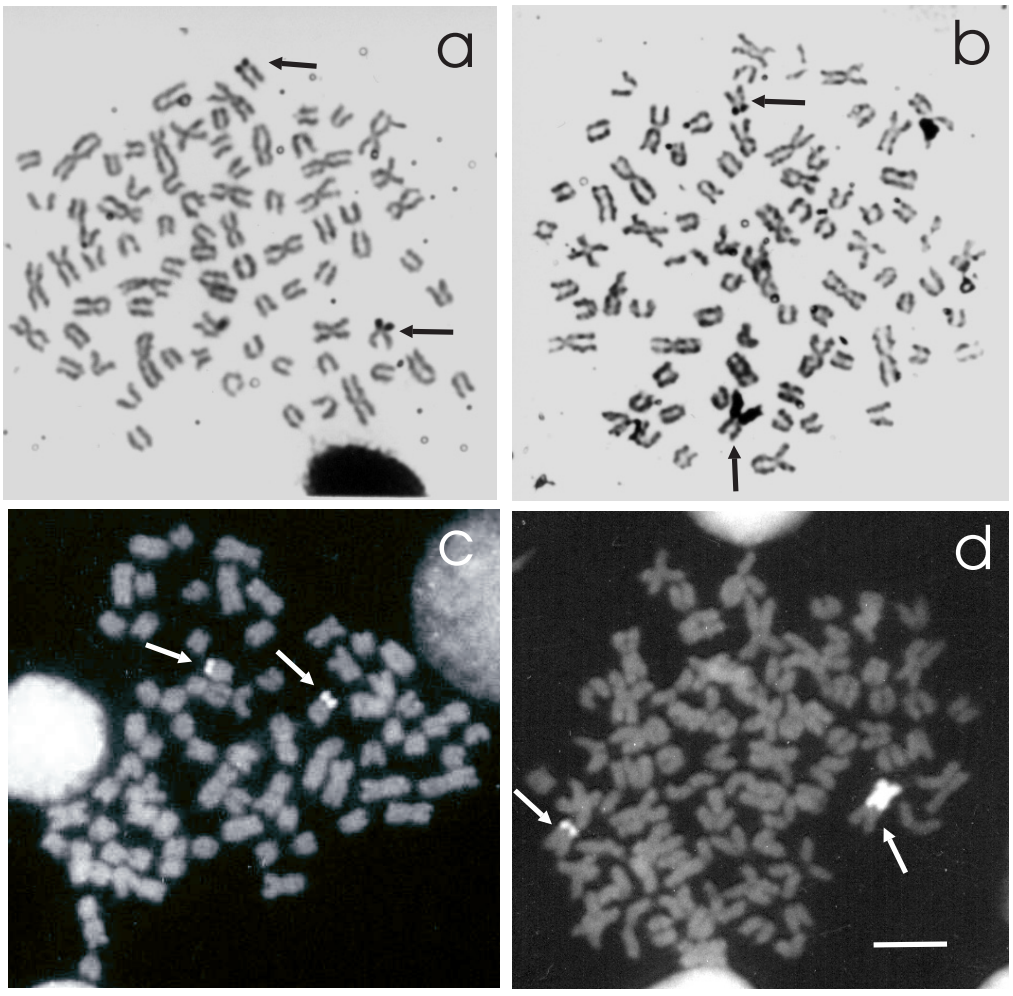


Fig. 3. Metaphase chromosomes of peled (*Coregonus peled*). a, b – stained with silver nitrate, c, d – stained with chromomycine $A_3$, a, c – homozygote of the NOR-bearing pair, b, d – heterozygote of the NOR-bearing pair. Arrows indicate nucleolar organizer region (NOR). Bar = 10 $\mu$m.

Table 1

Summarized data on chromosome studies of *Coregonus peled* (2n – diploid chromosome number; m – metacentric chromosomes, sm – submetacentric chromosomes; st – subtelocentric chromosomes; a – akrocentric chromosomes; NF – chromosome arm number)

| Species | 2n | m-sm | st | a | NF | References |
|---|---|---|---|---|---|---|
| *C. peled* (sensu Svärdson) | 80 | 12-18 | | 62-68 | 92-98 | NYGREN *et al*. 1971 |
| *C. peled* | 80 | 10-12 | | 68-70 | 90-92 | VIKTOROVSKIJ 1964 |
| | 74 | 22 | | 52 | 96 | KAYDANOVA 1978 |
| | 74 | | | | 96 | ANDRYASHEVA 1982 |
| | 76 | 22 | | 54 | 98 | JUNTUNEN 1987 |
| | 74-76 | 20-22 | | | 96 | KAYDANOVA 1989 |
| | 76 | 20 | 2 | 54 | 96 | present paper |

Table 2

Diploid chromosome number (2n) and chromosome arm number (NF) in peled (*Coregonus* peled)

| Individual | Number of metaphase chromosomes | | | | | | | | | | | | | | | | | | | | | | | Modal 2n | NF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <60 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | >80 | | |
| Cp1 | | | | | | | 2 | 2 | | | | | | 1 | 1 | 6 | 5 | 9 | | | | | | 76 | 96 |
| Cp3 | | | | | | | | | | | | | | 1 | 1 | 1 | | 9 | | | | | | 76 | 96 |
| Cp4 | | | | | | | | | | | | | 1 | | 1 | 2 | 1 | 7 | | 1 | | | | 76 | 97 |
| Cp5 | | | | | | | | | | | | | 1 | 1 | 2 | 2 | 1 | 5 | | | | | | 76 | 96 |
| Cp6 | | | | | | | | | | | | 1 | 1 | 3 | 1 | 1 | 3 | 11 | 1 | | | | 1 | 76 | 96 |
| Cp7 | | | | | | 1 | | | | | | 2 | 1 | 1 | 1 | 8 | 3 | 13 | | | 1 | | | 76 | 96 |
| Cp8 | | | | | | | | | | | | | 1 | | | 3 | 1 | 7 | | | | | | 76 | 96 |
| Cp9 | 1 | | | | | | | | | | | | 1 | 2 | | | 1 | 7 | 1 | | 1 | | | 76 | 96 |
| Cp10 | | | 1 | | | | | | 1 | | | | | | | 1 | 2 | 17 | | | | | | 76 | 96 |
| Cp11 | | | | | | | | | | | | | | 1 | 1 | 1 | 3 | 5 | | | | | | 76 | 96 |
| Cp13 | | | | | | | | | | | 1 | 2 | 1 | 3 | 4 | 5 | 3 | 10 | 1 | | | 1 | | 76 | 96 |
| Cp16 | | | | | | | | | | | 1 | | | | 2 | | 3 | 11 | | | | | | 76 | 97 |
| Cp17 | | | | | | | | | | | | | | | | 2 | 3 | 5 | | | | | | 76 | 96 |
| Pel1 | | | | | | | | | | | | 1 | | 4 | 1 | 11 | 2 | 14 | 2 | 4 | 1 | | | 76 | 97 |
| Pel2 | | | | | | | | | | | | 1 | | 2 | 1 | 8 | 2 | 17 | 2 | 1 | | | | 76 | 96 |
| Pel3 | | | | | | 1 | | | | | | | 2 | 1 | | 2 | 3 | 13 | 1 | | | | | 76 | 97 |
| Pel4 | | | | | | | | | | | | | | 2 | 1 | 8 | 4 | 19 | 3 | 3 | | | | 76 | 96 |
| Pel5 | | | | | | | | | | | | | | | 2 | 2 | 4 | 20 | 4 | 2 | | | | 76 | 96 |
| Pel6 | 1 | | | | | | | | | | | | | | | | 2 | 4 | | | | | | 76 | 96 |
| Pel7 | | | | | 1 | | | | | | | 1 | 1 | 1 | | 6 | 5 | 3 | | | | | | 74 | 96 |
| Pel8 | | 1 | | | | | | | | | 2 | 1 | | 2 | | 4 | 3 | 20 | 3 | 2 | | | | 76 | 96 |
| Pel13 | | | | | | | | | | | | | | 1 | 1 | 2 | 2 | 6 | | 2 | | | | 76 | 97 |
| Pel14 | | | | | | | | | | 2 | 1 | | | 1 | | 1 | 2 | 4 | 2 | | | | | 76 | 96 |
| Pel15 | | | | | | | | | | | 1 | | | 1 | | 3 | 1 | 7 | 1 | | | | | 76 | 96 |
| Pel44 | | | | | | | | | | | | 2 | | 1 | | 2 | | 3 | | | | | | 76 | 96 |
| Pel49 | 1 | | | | | | | | | | | | | | 1 | 2 | | 5 | | | | | | 76 | 96 |
| Σ | 3 | 1 | 1 | | 1 | 2 | 2 | 2 | 1 | 2 | 6 | 11 | 10 | 29 | 21 | 83 | 59 | 251 | 21 | 15 | 3 | 1 | 1 | | |

The location of the NORs in peled was investigated only by JANKUN *et al*. (2001), and the authors found one pair of NOR-bearing chromosomes (Fig. 3).

The location of the NOR is the same as observed by KAYDANOVA (1989). This author used only Giemsa staining but the NOR is clearly visible on one chromosome pair as an achromatic region. In analysed slides of five specimens, an arm length polymorphism on the NOR bearing chromosomes was observed (Fig. 3d ). NORs are the regions containing rRNA (5.8S, 18S and 28S) coding genes, on the base of which, during interphase, the nucleolus originates (SUMNER 1990). There are three kinds of polymorphism in the NOR sites in fishes:

– size polymorphism due to an amplification/deletion mechanism (NORs located on homologous

chromosomes are different in size) (ALONSO *et al*. 1999; JANKUN *et al*. 2003),

– translocation or transposition of the NOR site to new locations (NOR present on one or both homologous chromosomes) (REED & PHILLIPS 1995; WOZNICKI *et al*. 2000),

– activity polymorphism (rRNA sequences are actively transcribed on only one of homologous chromosomes) (AMEMIYA & GOLD 1986; CASTRO *et al*. 2001).

The first type of polymorphism was observed in the karyotype of peled investigated in the present work. Size differences between homologous NORs have been found in many fish species and other vertebrates (SWITONSKI *et al*. 1997; JANKUN *et al*. 2003). SCHMID (1982) reported a high frequency of duplications in Anura, always in a heterozygous condition. A similar situation was observed in brown trout (SANCHEZ *et al*. 1990). Our results in peled also indicate an important incidence (27%) of heteromorphism in the short arm of the NOR-bearing chromosome pair, which probably is due to NOR duplications. Amplification or deletion of NOR sites could be an effect of the crossing-over disorders caused by wrong meiotic conjugation between repetitive nucleotide sequences of homologous chromosomes (SWITONSKI *et al*. 1997).

Native Coregoninae are threatened by introgression with the peled gene pool (LUCZYNSKI *et al*. 1999). This study extends knowledge on karyotype characterictics and its variability in this relatively new species in Polish waters. The next step in the study of peled should be done by using molecular techniques, which could help find new cytogenetic markers. These methods can also be helpful in indicating the way the genome is reorganised in hybrid populations.

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