Japanese Journal of Ichthyology
Vol. 37, No. 4 1991

魚類学雑誌
37巻4号 1991年

Karyological Study of the Dolly Varden *Salvelinus malma* from Alaska

Takayoshi Ueda[1], Hiroyuki Fukuda[2] and Jindo Kobayashi[1]

[1] Department of Biology, Faculty of Education, Utsunomiya University, 350 Mine, Utsunomiya 321, Japan
[2] Mibu High-school, 1194 Fujii, Mibu 321-02, Japan

Abstract The karyotype of the dolly varden *Salvelinus malma* from Alaska was analysed. A pair of huge acrocentrics, which could have resulted from either tandem fusion or centric fusion followed by pericentric inversion, was observed. Ag-NORs were observed at the terminal regions of the second largest chromosome pair, and individual size-differences in Ag-NORs and in chromosomes with Ag-bands were found. From a comparison of the karyotypes of the dolly varden from Alaska and Hokkaido (Japan), karyotype differentiation by pericentric inversion and translocation was discussed.

There are more reports concerned with karyotype differentiation in salmonid fishes than in other fishes. Robertsonian-type variations within species and individuals have been reported by several investigators (Ohno et al., 1965; Roberts, 1968; Fukuoka, 1972; Thorgaard, 1976; Ueda et al., 1983; Ueda and Ojima, 1984a; Phillips and Kapuscinksi, 1987). Chromosome changes due to tandem fusion (Ueda and Ojima, 1983a, 1984b) and an increase or decrease in constitutive heterochromatin (Ueda and Ojima, 1983b; Ueda et al., 1985) have also been reported, as has intraspecific variation in both the number and locations on the chromosome of nucleolar organizer regions (NORs) (Ueda et al., 1988; Phillips et al., 1988, 1989a).

This report presents C- and Ag-banding karyological pictures of the dolly varden *Salvelinus malma* from Alaska (U.S.A.), and from a comparison with the karyotype of Hokkaido specimens (Ueda and Ojima, 1983a), discusses karyotype differentiation.

Materials and methods

Twelve eyed-stage embryos of the dolly varden *Salvelinus malma* from the Kubo Private Laboratory, originally obtained from Juneau, Alaska, U.S.A., were used in the present study. The sex was not checked. Chromosome slides were made by short-term-culture and direct methods (Ueda, 1986). A Giemsa C-banding technique following Sumner (1972) was used for detailed chromosome analyses. The silver banding technique of Howell and Black (1980) was used for staining NORs.

Results

Fig. 1 shows the C-banding karyotype of dolly varden ($2n=82$), which consists of 16 meta- (M) and submetacentrics (SM) and 66 acrocentrics (A). On this basis it is clear that NF (nombre fondamental) is 98. This karyotype form is characterized by a pair of huge A with intense C-bands at the centromere and at one point on the middle region of the long arm (a in Fig. 1), and a pair of large A with large, intense C-bands near the centromere (b in Fig. 1). In addition, C-bands are located on the terminal regions of some chromosomes.

Ag-NORs were observed on the terminal regions of the second largest chromosome pair (underlined in Fig. 2). Size-differences in Ag-NORs (silver stained NORs) and in chromosomes with Ag-bands were found among individuals. A pair of chromosomes with Ag-NORs had three different forms: large M with large Ag-NORs (LL-type), large M with small Ag-NORs (LS-type), and small SM with small Ag-NORs (SS-type). Four types of chromosome pairs, namely LL·LS-type (3 individuals), LL·LL-type (3 individuals), LL·SS-type (5 individuals), and LS·SS-type (1 individual), were recognized in each individual (Fig. 3).

Discussion

Dolly varden from Juneau, Alaska, had the same diploid chromosome number ($2n=82$) as examples from Niseko, Hokkaido, reported by Ueda and

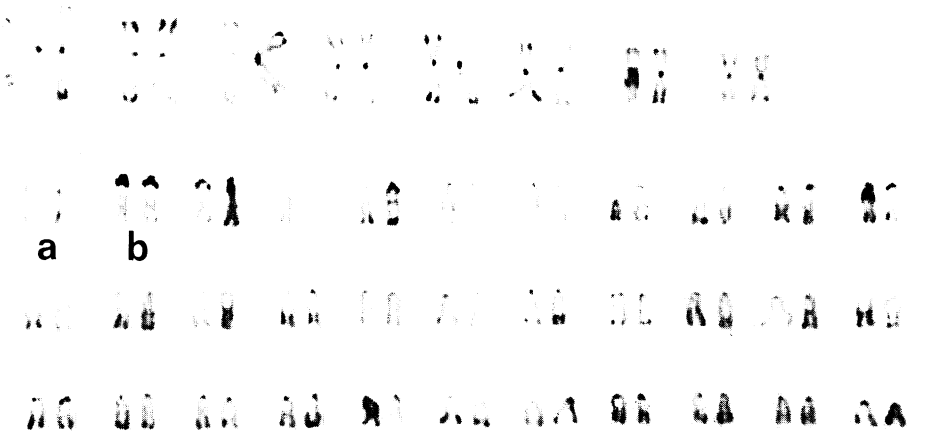

Fig. 1. The C-banding karyotype of the dolly varden from Juneau, Alaska. a, a pair of huge A with intense C-bands at the middle region of the long arm; b, a pair of large A with intense C-bands near the centromere.

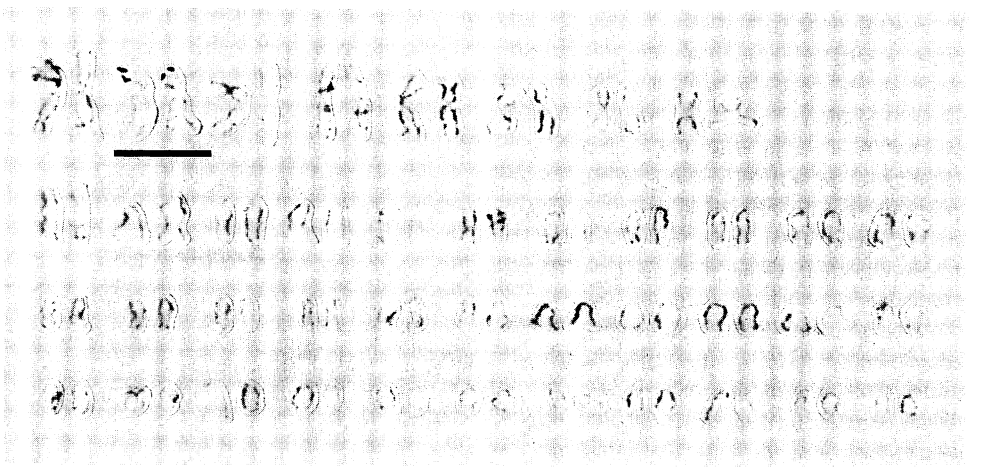

Fig. 2. The Ag-banding karyotype of the dolly varden from Juneau, Alaska. The underline indicates a pair of M or SM with Ag-NORs at the terminal region.

Ojima (1983a). Both populations of dolly varden had a pair of huge A. Diploid chromosome numbers of the dolly varden are two less than those of other species in the genus *Salvelinus* which lack huge A. Therefore, such huge A could have resulted from either tandem fusion or centric fusion followed by pericentric inversion.

A pair of large A with large, intense C-bands near the centromere was found in the fish from Juneau instead of a pair of M or SM with a structure similar to the satellite and intense C-bands on the short arm observed in the fishes from Niseko ("M or SM;

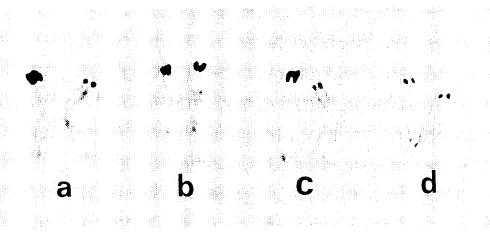

Fig. 3. Four types of the second largest chromosome pair with Ag-NORs found in the dolly varden from Juneau, Alaska. a, LL·LS-type; b, LL·LL-type; c, LL·SS-type; d, LL·SS-type.

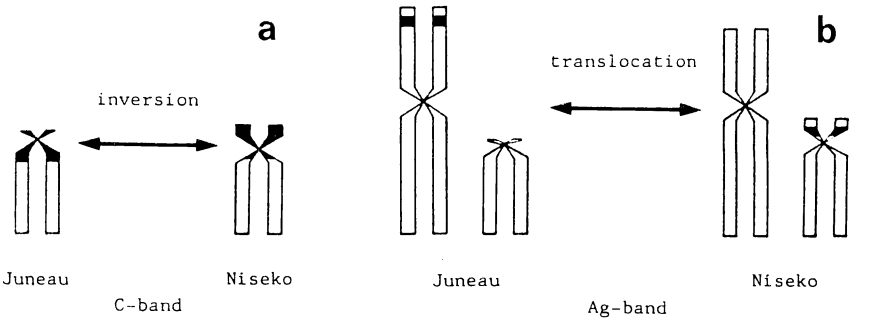

Fig. 4. A schematic figure of possible chromosome changes based on comparison between the dolly varden from Juneau, Alaska and Niseko, Hokkaido.

Niseko"). Because the length and size of the C-bands on both chromosomes are similar, their differences might have resulted from a pericentric inversion (Fig. 4).

Juneau dolly varden lacked a chromosome similar to the "M or SM; Niseko" type, and Ag-NORs were observed on terminal regions in a pair of large M or SM. The number of M and SM in Juneau was the same as that in Niseko, except "M or SM; Niseko". Therefore, both chromosomes with Ag-NORs may exist under the relation of a translocation (Fig. 4).

Individual differences in size of Ag-NORs were noted. Phillips et al. (1989b) observed NORs at similar positions on the chromosomes of Alaskan dolly varden, but individual size-differences of NORs have not been reported. Generally speaking, rDNA content and silver staining intensity are closely correlated (Warburton and Henderson, 1979). This size-difference could have resulted from unequal crossing during meiotic division or from a duplication of rDNA. Similar intraspecific heteromorphism in NOR-size has been reported in other fishes (Howell and Black, 1979; Foresti et al., 1981; Gold, 1984; Ueda et al., 1985; Takai and Ojima, 1986). We have also observed size-differences in Ag-NORs in the rainbow trout *Oncorhynchus mykiss* (=*Salmo gairdneri*), such differences being in accord with Mendelian inheritance (unpublished). Should such polymorphisms be regular, they might be useful as markers in biotechnological research, but more investigation is required to confirm this.

To date, individual polymorphisms of Ag-NORs; LL·LS, LL·LL, LL·SS, and LS·SS-type, have been found. A similar heteromorphic pair of chromosomes has been reported in the lake trout *Salvelinus namaycush* and has been discussed as possible sex chromosomes (Phillips and Ihssen, 1985). In addition, the existence of possible heteromorphic sex chromosomes in the rainbow trout (Thorgaard, 1977; Ueda and Ojima, 1984 a), sockeye salmon *Oncorhynchus nerka* (Thorgaard, 1978), and kokanee *Oncorhynchus nerka* (Ueda and Ojima, 1984c) has been reported. Makino (1932) and Galgano (1938) believed that, inasmuch as teleosts occupied a rather low position on the evolutionary ladder, the degree of difference between their sex chromosomes and autosomes was negligible. Future detailed chromosome analyses of salmonid fishes may bring new information that will permit a more complete view of sex chromosome differentiation.

Acknowledgments

We are very grateful to Dr. Tatsuro Kubo, The Kubo Private Laboratory, and Dr. Mineo Saneyoshi, Department of Biological Sciences, The Nishi-Tokyo University, for their proffer of materials for the present study.

Literature cited

Foresti, F., L. F. Almedia Toledo and S. A. Toledo. 1981. Polymorphic nature of nucleolus organizer regions in fishes. Cytogenet. Cell Genet., 31: 137–144.

Fukuoka, H. 1972. Chromosomes of the sockeye salmon (*Oncorhynchus nerka*). Japan. J. Genet., 47: 455–458.

Galgano, H. 1938. Le gametogenesi dell teleostems. Atti Soc. Ital. Anat., (7): 15–20.

Gold, J. R. 1984. Silver-staining and heterochromatin of chromosomal nucleolus organizer regions in North American cyprinid fishes. Copeia, 1984(1): 133–139.

Howell, W. M. and D. A. Black. 1979. Location of the nucleolus organizer regions on the sex chromosomes of the banded killifish, *Fundulus diaphanus*. Copeia, 1979(3): 544–546.

Howell, W. M. and D. A. Black. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1 step method. Experientia, 36: 1014–1015.

Makino, S. 1932. The chromosomes of fishes. Proc. Japan Acad., 8: 20–26.

Ohno, S., C. Stenius, E. Faisst and M. T. Zenzes. 1965. Post-zygotic chromosomal rearrangements in rainbow trout (*Salmo irideus* Gibbons). Cytogenetics, 4: 117–129.

Phillips, R. B. and P. E. Ihssen. 1985. Identification of sex chromosomes in lake trout (*Salvelinus namaycush*). Cytogenet. Cell Genet., 39:14–18.

Phillips, R. B. and A. R. Kapuscinski. 1987. A Robertsonian polymorphism in pink salmon (*Oncorhynchus gorbuscha*) involving the NOR region. Cytogenet. Cell Genet., 44: 148–152.

Phillips, R. B., K. A. Pleyte and S. E. Hartley. 1988. Stock-specific differences in the number and chromosome positions of the nucleolar organizer regions in arctic char (*Salvelinus alpinus*). Cytogenet. Cell Genet., 48: 9–12.

Phillips, R. B., K. D. Zajicek and P. E. Ihssen. 1989a. Population differences in chromosome-banding polymorphisms in lake trout. Trans. Amer. Fish. Soc., 118: 64–73.

Phillips, R. B., K. A. Pleyte and P. E. Ihssen. 1989b. Patterns of chromosomal nucleolar organizer region (NOR) variation in fishes of the genus *Salvelinus*. Copeia, 1989(1): 47–53.

Roberts, F. L. 1968. Chromosomal polymorphisms in North American land locked *Salmo salar*. Can. J. Cytol., 10: 865–875.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res., 75: 304–306.

Takai, A. and Y. Ojima. 1986. Some features on nucleolus organizer regions in fish chromosomes. Pages 899–909 *in* T. Uyeno et al., eds. Indo-Pacific fish biology. Ichthyological Soc. of Japan.

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

Thorgaard, G. H. 1977. Heteromorphic sex chromosomes in male rainbow trout. Science, 196: 900–902.

Thorgaard, G. H. 1978. Sex chromosomes in the sockeye salmon: Y-autosome fusion. Can. J. Genet. Cytol., 20: 349–354.

Ueda, T. 1986. Preliminary examination of obtaining chromosome preparation for fish breeding. Bull. Fac. Educ. Utsunomiya Univ., 36: 81–85.

Ueda, T. and Y. Ojima. 1983a. Karyotypes with C-banding patterns of two species in the genus *Salvelinus* of the family Salmonidae. Proc. Japan Acad., 59B: 343–346.

Ueda, T. and Y. Ojima. 1983b. Geographic and chromosomal polymorphisms in the Iwana (*Salvelinus leucomaenis*). Proc. Japan Acad., 59B: 259–262.

Ueda, T. and Y. Ojima. 1984a. Sex chromosomes in the kokanee salmon *Oncorhynchus nerka*. Bull. Japan. Soc. Sci. Fish., 50(9): 1495–1498. (In Japanese.)

Ueda, T. and Y. Ojima. 1984b. Karyological characteristics of the brown trout, the Japanese char and their hybrids. Proc. Japan Acad., 60B: 249–252.

Ueda, T. and Y. Ojima. 1984c. Sex chromosomes in the rainbow trout *Salmo gairdneri*. Bull. Japan. Soc. Sci. Fish., 50(9): 1499–1504. (In Japanese.)

Ueda, T., Y. Ojima, T. Kato and Y. Fukuda. 1983. Chromosomal polymorphisms in the rainbow trout (*Salmo gairdneri*). Proc. Japan Acad., 59B: 168–171.

Ueda, T., Y. Ojima and K. Naka. 1985. Chromosomal polymorphisms in the Biwa trout (Salmonidae) with an increase or decrease in constitutive heterochromatin. Proc. Japan Acad., 61B: 483–485.

Ueda, T., R. Sato and J. Kobayashi. 1988. Silver-banded karyotypes of the rainbow trout, the brook trout and their hybrids: disappearance in allotriploids of Ag-NORs originated from the brook trout. Japan. J. Genet., 63: 219–226.

Warburton, D. and A. S. Henderson. 1979. Sequential silver staining and hybridization *in situ* on nucleolus organizing regions in human cells. Cytogenet. Cell Genet., 24: 168–175.

(Received July 8, 1990; accepted November 13, 1990)

アラスカ産オショロコマの核学的研究

上田高嘉・福田博之・小林仁道

アラスカ産オショロコマ *Salvelinus malma* の C- および Ag-バンド核型分析を行った．染色体数は 2n＝82 で，M および SM 染色体が 16 本，A 染色体が 66 本であった．動原体部および長腕の中間部に C-バンド濃染を持ち，2 本の A 染色体の縦列結合あるいは動原体融合に続く逆位によって生じたと推定される 1 対の大型 A 染色体，および動原体付近に大きな C-バンド濃染を持つ 1 対の大型 A 染色体が認められた．Ag-NORs は 2 番 M 染色体の端部に存在した．この M 染色体が異形対である個体が認められ，性染色体の可能性が考えられた．また，個体間に Ag-バンドの大きさの多型がみとめられた．北海道産オショロコマとの比較において，大きな C-バンドを持つ染色体の形態および Ag-バンドの位置などに相違が認められ，転座および逆位の関わりが推定された．

（上田・小林：321 宇都宮市峰町 350　宇都宮大学教育学部生物学教室；福田：321-02 壬生町藤井 1194　壬生高等学校）