

Chromosomes of Offspring Resulting from Crossing Coho Salmon and Brook Trout

Teruya Uyeno

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Abstract Chromosome numbers of the hybrids between *Oncorhynchus kisutch* ($2n=60$) and *Salvelinus fontinalis* ($2n=84$) were $2n=60$ in the cross *O. kisutch*♀ × *S. fontinalis*♂, and $2n=72$ in the reciprocal cross. The karyotype of offspring from the former cross was identical with that of the female parent, but the karyotype of offspring from the reciprocal cross was intermediate of karyotypes of both parents. The karyotype of *S. fontinalis* looked as most primitive among karyotypes of salmonid fishes ever reported. The evolution of the salmonid karyotype is also briefly discussed.

Though the history of chromosome studies of salmonid fishes can be traced back more than 40 years, firmly reliable karyotypes for comparative studies appeared only after the technique of the somatic chromosome squash was developed for fishes around 1965. As far as I am aware, the earliest, good photomicrographs of salmonid chromosomes are by Nygren et al. (1968b), for Atlantic salmon, *Salmo salar*.

Karyotypes of interspecific hybrids between two species of salmonids, chum salmon, *Oncorhynchus keta*, and kokanee salmon, *O. nerka*, were reported by Sasaki et al (1968), with good photomicrographs. In their study, the hybrids of both reciprocal crosses were found to have a karyotype exactly intermediate ($2n=66$) between *O. keta* (74) and *O. nerka* (58).

In 1969 and 1970, intergeneric crosses of coho salmon, *Oncorhynchus kisutch* (Walbaum), and brook trout, *Salvelinus fontinalis* (Mitchill), successfully produced offspring at the Grayling and Thompson Research Stations of the Department of Natural Resources in the State of Michigan, U. S. A., under the direction of Dr. Leonard N. Allison.

The purpose of this paper is to report on the karyotypes of the crosses made in 1970, and thereby to enhance the understanding of the nature of the hybridization and to discuss some

aspects of their karyotypic changes in the family Salmonidae.

Material and methods

Eight specimens of coho salmon, 10 specimens of brook trout, 14 specimens of offspring from cross between female coho salmon and male brook trout, and 10 specimens from the reciprocal cross were used for this study, but only 4, 6, 12 and 5 specimens, respectively, provided appropriate chromosome spreads (Fig. 1). All specimens used and parental specimens of both intergeneric crosses are deposited in the fish collection of the University of Michigan, Museum of Zoology. The parental specimens were all obtained in Michigan, although the original stock of coho salmon was brought from the Pacific coast to Lake Michigan. Dr. Allison, Mr. Warren Yoder, and Mr. Keith M. Pratt actually produced the artificial crosses.

In the cross between female coho salmon and male brook trout, about 2,450 eggs were fertilized, and several hundred specimens were still surviving in March, 1971. In the reciprocal cross, about 1,200 eggs were fertilized, but only about 50 specimens were surviving in the beginning of March, 1971. According to Dr. Allison, usually 60 to 80 percent of ferti-

lized eggs survive in cases of intraspecific fertilization at the two stations.

The chromosome preparations were made following the technique described in detail by Beamish (1970), with some minor modifications. Specimens were injected with 0.05% Velban (Lillie Co., Ltd.) about 6 hours prior to the removal of gill arches, which were placed in distilled water for about one hour. Gills were fixed and stained by 4% acetic orcein (in 60% glacial acetic acid).

Results

Comparison of the karyotypes led to rather surprising results (Table 1). The diploid chromosome numbers were 60 for coho salmon and 84 for brook trout. The hybrids between female brook trout and male coho salmon had a diploid number of 72, intermediate between their parental species and thus indicating receipt of a haploid chromosome set from each parent. But the fish produced from the reciprocal cross, female coho salmon \times male brook trout, had the chromosome number and form identical with that of the coho salmon (Fig. 2). This strongly suggests that these offspring from the latter combination are the result of parthenogenesis, especially of gynogenetic reproduction.

Cases of gynogenetic reproduction in nature have been known among fishes for many years, as summarized by Schultz (1969), and recently *Carassius auratus gibelio* and *Carassius auratus langsdorffii* were found to be gynogenetic (Cherfas, 1966; Kobayashi et al., 1970; Kobayashi, 1971; Kobayashi and Ochiai, 1972). These examples are, however, found to be caused by triploidy that was probably originated by hybridization. The case of the cross between the female coho salmon and the male brook trout is the result of artificial insemination, but it is unusual that the reciprocal cross produced the true hybrids.

Discussion

It seems that the theory of the origin of the

salmonid karyotype by polyploidy is now well established by the supporting evidences of the numbers of chromosome and chromosome arms, the presence of multivalents in meiosis, the amount of DNA, and the number and characters of isozymes (Nygren et al., 1968; Ohno et al., 1969; Numachi et al., 1972). A similar case is also reported for the origin of the karyotype of the fish family Catostomidae (Uyeno and Smith, 1972). On the basis of these data, Ohno et al., (1969) stated that the diploid ancestor of the Salmonidae must have had a diploid complement as well as a DNA value similar to that of the present day anchovy (Engraulidae).

In the recent classification of teleostean fishes the Clupeiformes which includes the Engraulidae are separated from the Salmoniformes at the superordinal level (Greenwood et al., 1966), or at the ordinal level (Gosline, 1971). So the closest relatives of the family Salmonidae in freshwater of the northern hemisphere are considered to be smelts (osmeroids), and pikes (esocoids). The reported chromosome number of osmerids ranges from $2n=48$ to 54 (Ohno et al., 1969; Nygren et al., 1971), and of *Esox lucius* is 50 (Nygren et al., 1968a), with all acrocentric chromosomes. My preliminary examination of the karyotypes of *Esox lucius* and *Esox americanus* in Michigan indicated that both have 50 acrocentrics, and all species of the Esocidae examined by Beamish et al. (1971) also had 50 acrocentrics.

The chromosomes of brook trout ($2n=84$) can be grouped into two distinct types (Fig. 2A): 16 large metacentric and submetacentric chromosomes and 68 small acrocentric chromosomes, yielding a total arm number of 100. This results agree well with the counts obtained by Davisson and Mayers (Benirschke and Hsu, 1971). If it is assumed that the large metacentrics were derived by Robertsonian fusions, the ancestral "tetraploid" form must had 100 acrocentrics, and the diploid ancestor had 50 acrocentrics as in karyotypes of the Esocidae. If the above assumption is correct, then the brook trout has one of the most generalized

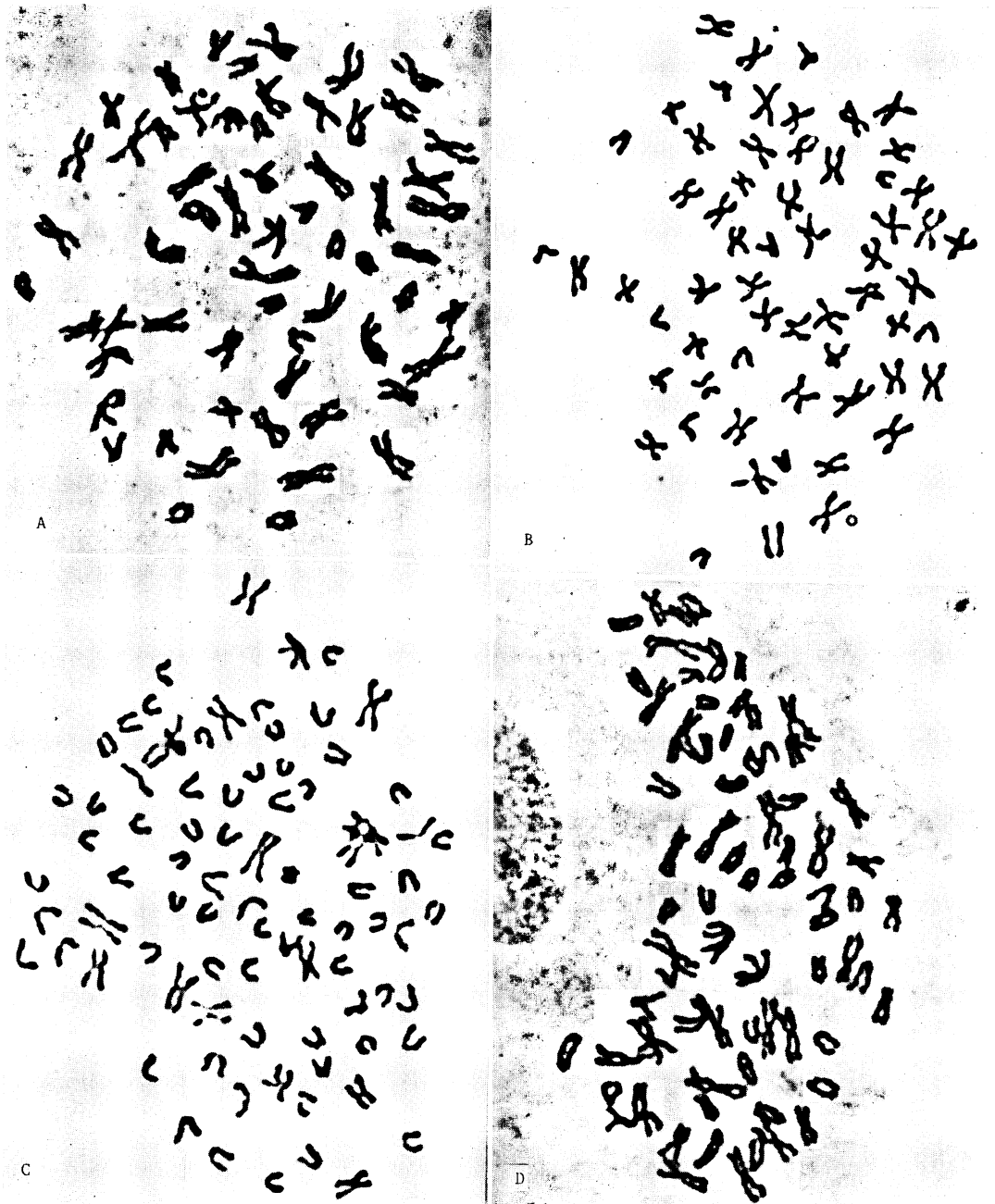


Fig. 1. Photomicrographs of chromosome spreads. A, hybrid ($2n=60$) of *Oncorhynchus kisutch* ♀ × *Salvelinus fontinalis* ♂; B, *O. kisutch* ($2n=60$); C, *S. fontinalis* ($2n=84$); D, hybrid ($2n=72$) of *S. fontinalis* ♀ × *O. kisutch* ♂.



Fig. 2. Karyotypes: (S.f.), *Salvelinus fontinalis*, $2n=84$; (O.k.), *Oncorhynchus kisutch*, $2n=60$; (O.k. x S.f.), *O. kisutch* ♀ x *S. fontinalis* ♂, $2n=60$; (S.f. x O.k.), *S. fontinalis* ♀ x *O. kisutch* ♂, $2n=72$.

Table 1. Chromosome data.

| Diploid numbers | Distribution of counts for the diploid chromosome number | | | | | | | | | | | | | | Total no. of cells observed |
|--|--|----|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------------|
| | 57 | 58 | 59 | 60 | 61 | 62 | 69 | 70 | 71 | 72 | 81 | 82 | 83 | 84 | |
| <i>Oncorhynchus kisutch</i> | 1 | 1 | 4 | 30 | | | | | | | | | | | 36 |
| <i>O. kisutch</i> ♀ × <i>S. fontinalis</i> ♂ | | 1 | 2 | 22 | 0 | 4 | | | | | | | | | 29 |
| <i>S. fontinalis</i> ♀ × <i>O. kisutch</i> ♂ | | | | | | | 1 | 2 | 2 | 19 | | | | | 24 |
| <i>Salvelinus fontinalis</i> | | | | | | | | | | | 3 | 3 | 2 | 18 | 26 |

karyotypes ever reported among salmonine fishes. Nygren et al. (1971) reported that *Salmo trutta* and *S. alpinus* have $2n=80$ and possess 20 metacentrics, and the arm number for both species is 100. Though figures of chromosomes of these species were not provided in their report, these two species also might have simple karyotype which was derived by Robertsonian fusions of 40 acrocentrics.

The deviations of the arm number from 100 are probably the result of some structural rearrangements by the shifting of the centromere position in some fused and unfused chromosomes, which reduced or increased the number of arms. The karyotype of *Salmo salar* suggests the former type, and the karyotypes of *Oncorhynchus kisutch*, *O. keta*, and *O. nerka* suggest the latter type of structural rearrangements.

The karyotype of coho salmon (Fig. 1) includes the following types of chromosomes: 38 metacentrics large to small in size, 4 large submetacentrics, 2 medium sized submetacentrics, 2 small submetacentrics, and 14 acrocentrics. In the arm number calculation, if I follow Sasaki et al. (1968) in designating very small arms as "m", the formula for the arm number becomes as follows:

$$(38+4+2) 2+(16+2\sim 6\text{ m})=104+2\sim 6\text{ m}.$$

(Number of very small arms is difficult to count exactly). This karyotype of coho salmon can not be easily derived from the karyotype of the hypothetical ancestor with 100 acrocentrics, as in the case of brook trout which has 16 definitely biarmed large metacentrics (indicative of fusion of 2 acrocentrics) and 68 acrocentrics as simply formulated as $(16)2+68=100$.

In order to derive the karyotype of coho salmon from the hypothetical ancestral salmonid karyotype with 100 acrocentrics, it has to be assumed that at least 2 pairs of small metacentrics (or submetacentrics) and 2 pairs of small chromosomes with shorter arms were derived by structural rearrangements rather than Robertsonian fusions.

In a recent work, Nygren et al. (1971) reported on the number of chromosomes, number of metacentric chromosomes, and N.F. values (arm number) for 6 species of salmonoid fishes. They included *Osmerus eperlanus* ($2n=54$) in the family Salmonidae, and stated that the species has the lowest chromosome number so far reported in the family. The genus *Osmerus*, however, has been placed in the separate family Osmeridae (Greenwood et al., 1966), or even in the separate suborder Osmeroidei (Weitzman, 1967), and should not be included in the Salmonidae, especially if all of salmonid fishes are "tetraploid", and osmerids are "diploid".

With these considerations, the results of the hybridization between species of two salmonid genera *Oncorhynchus* and *Salvelinus* are rather remarkable suggesting the need for further cytogenetic investigation.

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Literature cited

- Beamish, R. J. 1970. Factors affecting the age and size of the white sucker *Catostomus commersoni* at maturity. Ph. D. thesis. Univ. Tronto, Tronto, Ontario. 170 pp.
- Beamish, R. J., M. J. Merrilees, and E. J. Crossman. 1971. Karyotypes and DNA values for members of the suborder Esocidae (Osteichthyes: Salmoniformes). *Chromosoma*, 34 : 436-447, figs. 1-12.
- Benirschke, K. and T. C. Hsu (Ed). 1971. Chromosome atlas: Fish, Amphibians, Reptiles and Birds. Vol. 1. i-ix, 52 folios.
- Cherfas, N. B. 1966. Natural triploidy in females of the unisexual form of the goldfish (*Carassius auratus gibelio* Bloch). *Genetika*, 5 : 16-24, figs. 1-6.
- Gosline, W. A. 1971. Functional morphology and classification of teleostean fishes. Univ. Press of Hawaii. 208 pp, 29 figs.
- Greenwood, P. H., D. E. Rosen, S. H. Weitzman, and G. S. Myers. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. *Bull. Amer. Mus. Nat. Hist.*, 131 : 339-456, figs. 1-9, pls. 21-23, charts 1-32.
- Kobayashi, H. 1971. A cytological study on gynogenesis of the triploid gimbuna (*Carassius auratus langsdorfi*). *Zool. Mag.*, 80(9) : 316-322, figs. 1-15.
- Kobayashi, H., Kawashima, and N. Takeuchi. 1970. Comparative chromosome studies in the genus *Carassius*, especially with a finding of polyploidy in the gimbuna (*C. auratus langsdorfi*). *Japan. J. Ichthyol.*, 17(4): 153-160, 4 figs. In Japanese.
- Kobayashi, H. and H. Ochiai. 1972. Chromosome studies of the hybrids, gimbuna (*Carassius auratus langsdorfi*) \times kinbuna (*C. auratus* subsp.) and gimbuna \times loach (*Misgurnus anguillicaudatus*). *Zool. Mag.*, 81(1) : 67-71, figs. 1-3.
- Numachi, K., Y. Matsumiya, and R. Sato. 1972. Duplicate genetic loci and variant forms of malate dehydrogenase in chum salmon and rainbow trout. *Bull. Japan. Soc. Sci. Fisher.*, 38(7) : 699-706, figs. 1-2.
- Nygren, A., P. Edlund, U. Hirsch, and L. Åhsgren. 1968a. Cytological studies in perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), pike-perch (*Lucioperca lucioperca* L.), and ruff (*Acerina cernua* L.). *Hereditas*, 59 : 518-524, figs. 1-20.
- Nygren, A., B. Nilsson, and M. Jahnke. 1968b. Cytological studies in Atlantic salmon (*Salmon salar*). *Annales Academiae Regiae Scientiarum Upsaliensis Kungl. Vetenskapssamhällets I. Uppsala Arsbok*. 12 : 21-52, figs. 1-30.
- Nygren, A., B. Nilsson, and M. Jahnke. 1971. Cytological studies in the smelt (*Osmerus eperlanus* L.). *Hereditas*, 67 : 283-286, figs. 1-7.
- Ohno, S., J. Muramoto, J. Klein, and N. B. Atkins. 1969. Diploid-tetraploid relationship in clupeoid and salmonoid fish. *Chromosomes Today*, 2 : 139-147, figs. 1, pls. 1-3.
- Sasaki, M., S. Hitotsumachi, S. Makino, and T. Terao. 1968. A comparative study of the chromosomes in the chum salmon, the kokanee salmon and their hybrids. *Caryologia*, 21(4) : 389-394, figs. 1-3.
- Schultz, R. J. 1969. Hybridization, Unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *American Naturalist*, 103 (934): 605-619, figs. 1-2.
- Uyeno, T. and G. R. Smith. 1972. Tetraploid origin of the karyotype of catostomid fishes. *Science*, 175: 644-646, fig. 1.
- Weitzman, S. H. 1967. The origin of the stomiatoid fishes with comments on the classification of salmoniform fishes. *Copeia*, 1967(3) : 507-540, figs. 1-18.
- (Nippon Luther Shingaku Daigaku, 3-10-20 Osawa, Mitaka-shi, Tokyo, Japan)

ギンザケ, カワマス, および両種間雑種の染色体

上野 輝弥

ギンザケ *Oncorhynchus kisutch* の染色体数は $2n=60$, カワマス *Salvelinus fontinalis* の染色体数は $2n=84$ であった。これら二種の属間雑種の染色体数はギンザケ \times カワマス σ の場合 $2n=60$, ギンザケ $\sigma\times$ カワマス σ の場合 $2n=72$ であった。前者の場合、核型は雌親と同じであったが、後者の核型は両親の核型の中間型であった。その染色体は雄親と雌親から1セットずつきたものと思われる。またカワマスの核型は2箇の染色体が合着してできたと思われる16箇の大きな中部着糸型の染色体と68箇の端部着糸型の染色体からなっており、その腕数は100である。これはサケ科核型の四倍体起源説からみて、これまでに報告されているサケ科核型中もっとも単純で原始的な型であると思われる。サケ科核型の祖先型はカワカマス科 *Esocidae* のように $2n=50$ で全ての染色体が端部着糸型であったとすれば、これが四倍体となり $2n=100$ となって、その後次第に合着により染色体数が減り、さらにある染色体では構造的再編成がおこって現生のサケ科魚類各種の核型が出現したのではないかと推論される。

(東京都三鷹市大沢 日本ルーテル神学大学)