p. 9

r. pict. w. blow out
n DNA - see Ann. Rev.

- great variability
alpine
- one pop. w/ polymorphism?

send to Cuellar?

Patterns of Chromosomal Nucleolar Organizer Region (NOR) Variation in Fishes of the Genus Salvelinus

Ruth B. Phillips, Kay A. Pleyte, and Peter E. Ihssen

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Abstract- The chromosomal locations of the nucleolar organizer regions (NORs) in two North American species of Salvelinus, S. confluentus and S. malma are reported. This brings the total number of Salvelinus species examined for chromosomal NORs to 6, and the total number of species of salmonid fishes examined for NORs to 17. Arrangement of the NOR differences into a phylogenetic hypothesis supports morphological, biochemical, and other cytogenetic data which group S. fontinalis with S. namaycush and S. malma with S. alpinus. The NOR data also groups S. leucomaenis with S. confluentis. The relationship between these two species and the other Salvelinus species remains unresolved.

Introduction:

The chromosomal location of the nucleolar organizer regions (NORs) of three of the five Salvelinus species of North America was determined previously by using chromomycin A3 (CMA3) staining and silver staining (Phillips and Ihssen, 1985). The chromomycin A3 (CMA3) technique stains active and inactive NORs in amphibians and fishes (Schmid, 1982; Gold and Amemiya, 1986). Although all the species in the related genera of Salmo and Oncorhynchus have only one chromosome pair with NORs (Phillips and Ihssen, 1985; Phillips, Zajicek and Utter, 1986), the NORs were found on several different chromosome pairs in these three Salvelinus species: S. namaycush (lake trout), S. fontinalis (brook trout) and S. alpinus (arctic char). In this paper the chromosomal location of the NORs in the other two species, S. malma (Dolly Varden char) and S. confluentus (bull trout), is described and a phylogenetic hypothesis for these species using this data is presented.

The five North American species of the genus Salvelinus have been assigned to three subgenera by Behnke (1965, 1980). These are the subgenus Cristovomer with S. namaycush, the subgenus Baione with S. fontinalis, and the subgenus Salvelinus, which includes the species in the arctic char complex. This group includes S. alpinus, which has a circumpolar distribution in the arctic, S. malma, which occurs sympatrically with S. alpinus in the north Pacific, and S. confluentus, which is found in the Rocky mountains (Cavender, 1980). In the Far East another species, S. leucomaenis (2n=84) is considered to be more closely related to S. namaycush by Savvaitova (1980) and Viktorovsky (1978), but

is placed in the subgenus Salvelinus by Behnke (personal communication). Behnke divides the subgenus Salvelinus into the S. alpinus- S. malma complex and a group comprising S. confluentus, S. leucomaenis and S. albus, which is found in the Kamchatka peninsula.

The possible phylogenetic relationships between the North American Salvelinus species were discussed in a paper by Cavender (1984), in which he summarizes his work and the work of others on the cytotaxonomy of Salvelinus. He presents two alternative cladograms based on chromosome number (2n) and chromosome arm number (NF). A major point of uncertainty is whether S. confluentus has a sister relationship to S. malma and S. alpinus, or is more closely related to S. fontinalis and S. namaycush and S. leucomaenis. The data on NORs provides some additional evidence relevant to this problem, which remains unresolved.

Materials and Methods:

Eggs of S. confluentus were obtained from fish in Arrow Lake, British Columbia by the Canadian Department of Fisheries and Oceans and eggs of S. malma were obtained from fish collected near Juneau, Alaska by the Alaska Fish and Game Department. Chromosome preparations were made from embryos from fertilized eggs using methods described previously (Phillips and Zajicek, 1982). Embryos of approximately 180 degree days were incubated in culture media with 25 ug/ml colchicine and fixed after 4 hours with 3:1 methanol acetic acid fixative. Chromosome slides were made according to the methods of Kligerman and Bloom (1977), in which fixed material was pulverized in 45% acetic acid and the material dropped onto heated slides. Slides were stained with the

silver staining procedure of Howell and Black (1980) to visualize the Ag-NORs, and with chromomycin A3 for 2 hours followed by counterstaining with distamycin A for 15 minutes for the CMA3 banding. Slides were viewed with a Zeiss Universal microscope on bright field for the Giemsa-stained and silver-stained slides and with a BG 12 excitation filter and 47 or 50 barrier filter for the CMA3 stained slides. Six figures were examined for each individual. Photographs were made using Kodak Technical Pan film with standard development, and 8 x 10 prints were made for karyotyping.

Results:

Location of NORs:

The locations of the NORs in S. confluentus and S. malma, were identified by silver staining and CMA3 staining. In these two species the NORs were found at one location on one chromosome pair in all individuals examined. There was a complete correspondence between NORs as identified by CMA3 staining and silver staining. In S. malma the NORs were found at a telomeric location on the largest submetacentric chromosome and in S. confluentus the NORs comprised the entire short arm of a large acrocentric chromosome (see Figure 1).

The locations of the NORs in five species of Salvelinus are illustrated in figure 1 and summarized in Table 1. In the other three species the location of the NORs was multi-chromosomal (Phillips and Ihssen, 1985). Considerable variation between individuals in the number of chromosomes with NORs was found in all three of these species, although the location of the NORs was constant for all of the cells of a

given individual. The total number of NORs per genome varied from 4-10 in S. fontinalis, 4-12 in S. namaycush and 2-6 in S. alpinus.

In each species with multichromosomal NORs some of the NORs were found at the same location in almost every individual, while other NORs were quite variable between individuals (Phillips et al., 1988a; Phillips et al., 1988b). The constant NOR sites were present on both members of homologous chromosomes, while the variable sites were often present on only one of the two homologous chromosomes. The constant site in S. alpinus was at the telomeres of the short arms of the largest submetacentric chromosome pair in the genome. The constant sites in S. fontinalis were on the short arms of two acrocentric chromosome pairs and in S. namaycush they were on the short arms of one acrocentric chromosome pair and the telomeres of another acrocentric chromosome pair.

Phylogenetic Analysis of NOR Character States:

Differences have been found between related fish species in the haploid number of chromosomal NORs, the specific chromosome(s) on which the NORs are located and the precise chromosomal location in the Salmoninae (Phillips and Ihssen, 1985; Phillips, Zajicek and Utter, 1986) and in cyprinid fishes (Gold, 1984; Gold and Amemiya, 1985).

In order to arrange NOR character states into a phylogenetic hypothesis, one must identify homology of character states between species and determine character state polarity. In order to determine character state polarity, the character states of species in related taxa must be examined so that the pleisomorphic character state can be

determined. The NOR phenotypes of 17 salmonid species analyzed to date are presented in Table 2. Fourteen of the seventeen species have the NOR on one chromosome pair, and nine including at least one species in each genus have the NOR on the short arms of an acrocentric chromosome (Type A in the Table). An additional four species have the NOR on the short arms of a submetacentric chromosome (Type A'). This is a minor change, because it is often simply the result of additional heterochromatin added to the region flanking the NOR on the short arm (Phillips and Hartley, 1988). Because NOR phenotype A is found in each genus including one species in the related Coregoninae (Phillips, unpublished data), we have assumed that this phenotype is pleisomorphic for the Salmoninae.

NOR phenotype A is also found in the majority of fishes and this type is also thought to be primitive for most vertebrates (Hsu et al., 1975; Schmid, 1978). If this is the case, then the initial ancestral tetraploid salmonid fish would have had 2 pairs of acrocentric chromosomes with the NORs on the short arms, and the NORs must have been consolidated to the short arms of one acrocentric chromosome pair shortly after tetraploidization. Studies of several tetraploid cyprinid fishes (Takai and Ojima, 1982) have revealed only one chromosome pair with NORs, suggesting that consolidation of NORs to one chromosome pair may be a common event in tetraploids.

If we consider NOR type A as the pleisomorphic NOR character for the Salmoninae, then S. confluentus and S. leucomaenis (Ueda and Ojima, 1983) would have the pleisomorphic NOR character state, and the other species in the genus Salvelinus would be placed into two groups which

share common derived character states (synapomorphies).

In order to define the synapomorphies precisely, a sequential method involving G banding for identification of chromosomes followed by CMA3 staining or CMA3 staining of meiotic chromosomes in hybrids would be required. Since a reliable G banding method was not available, chromosomes were divided into groups on the basis of size and centromere position.

S. fontinalis and S. namaycush have a very similar pattern with a constant NOR site on the short arms of the largest acrocentric chromosome pair and additional NORs on 5-12 different chromosome pairs in three different types of locations: short arms of acrocentric pairs, telomeres of acrocentric pairs and telomeres of metacentric pairs. The only NOR in S. confluentis is on the largest acrocentric pair, which the similar in size to the acrocentric chromosome with the constant NOR found in S. fontinalis and S. namaycush. S. malma and S. alpinus share a synapomorphy since the only NOR chromosome pair in S. malma and the constant NOR chromosome pair in the North American S. alpinus is the largest submetacentric chromosome in the karyotype. In order to be completely certain of this synapomorphy, homology of the two chromosomes would have to be shown using G banding, which has not yet been accomplished. However, the karyotypes of these two species are quite similar to one another (Cavender, 1984), so this is a plausible assumption. One possible cladogram summarizing all of the chromosome data is shown in figure 2.

Discussion:

Examination of the NOR character states in the Salvelinus species supports the other chromosome data which indicate a major split between S. namaycush and S. fontinalis on the one hand and S. alpinus and S. malma on the other. Analysis of the other karyotype data suggests that S. leucomaenis from Japan should be grouped with S. fontinalis and S. namaycush, since all three species have 2n=84, NF=100, and 8 metacentric chromosome pairs which are all larger than the 34 acrocentric pairs, a karyotype considered primitive by Cavender. (NF= number of chromosome arms) However, if we are correct in assuming that S. leucomaenis also has the primitive NOR character state, its placement with respect to the other species cannot be resolved using NOR data alone. There is some question about this, since the location of NORs in the karyotype of S. leucomaenis was deduced from the description by Ueda and Ojima (1983) of satellites identical to those found by us on the large acrocentric chromosome pair with NORs in S. confluentus, and not by direct CMA3 or silver staining. The three other species have derived karyotypes, with 2n=78, NF=102, and 12 metacentric pairs for S. confluentus, 2n=78, NF=98 and 10 metacentric pairs for S. alpinus, and 2n=82, NF=98 and 8 metacentric pairs for the southern form of S. malma in North America. The northern form of S. malma in North America has not been karyotyped. A close relationship between S. alpinus and S. malma is suggested since they share a derived large acrocentric chromosome, which is twice as large as the others or any of the acrocentrics in the other Salvelinus species.

Cavender (1984) suggests that the closest relative to S. confluentus

may be S. kronicus (also called S. albus) from the Kamchatka River basin, which was reported to have 2n=78 and NF=100 with 11 metacentric chromosome pairs. Our results from the karyotyping of S. confluentus from Arrow Lake, British Columbia, suggest that S. confluentus and S. kronicus may have identical karyotypes, since silver staining reveals that the 12th metacentric pair in the karyotype of S. confluentus prepared by Cavender is actually a large acrocentric chromosome pair with the NOR comprising its entire short arm.

The chromosome data support the morphological data, which suggest that there are 5 distinct species in North America subdivided into three groups: one with S. fontinalis and S. namaycush, another with S. malma and S. alpinus, and a third group with S. confluentus. A recent electrophoretic survey of protein loci in the five species (Leary, et al., personal communication) found that S. malma and S. alpinus shared alleles at all loci, while the other three species were distinct from each other and the S. malma-S. alpinus complex.

The nucleolar organizer regions are the sites of the 18S and 28S ribosomal RNA genes in animal chromosomes (Hsu, et al., 1975). The genes for the 18S and 28S ribosomal RNA are present in multiple copies of a repeating unit which contains both rapidly evolving spacer regions and conserved coding regions. Comparison of DNA sequences between the different species should yield many more characters for phylogenetic analysis. Preliminary restriction maps of the rRNA cistrons in S. namaycush and S. fontinalis have been prepared (Popodi et al., 1985), and we are preparing maps for the other Salvelinus species. We plan to identify phylogenetically informative regions in these cistrons and then

do fine structure restriction mapping or sequencing of selected regions in order to obtain multiple characters for a phylogenetic analysis of Salvelinus using rDNA.

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Table 1. Number of NORs per Diploid Genome in Different Salvelinus Species from North America

Species	Chromosomal Location of NORs				Total NORs
	Acrocent. Short Arms	Acrocent. Telomeres	Submetacent. Telomeres	Metacent. Telomeres	
S. confluentus (12)	2	--	---	---	2
S. fontinalis (20)	2-8*	0-2	--	0-1	4-10
S. namaycush (91)	2-6*	2-4*	--	0-2	4-12
S. alpinus (60)	--	0-2	2*	0-3	2-6
S. malma (12)	--	--	2	--	2

Numbers indicate the range of NORs found in different individuals of a given species. *Indicates major NOR sites in species with NORs at more than one chromosomal location. The number of individuals examined for each species are in parentheses.

Table 2. Number and Chromosomal Location of NORs in Different Salmonid Species

Species	Common Name	Major NORs Number/2N	Major NORs Location*	Minor NORs Number	Minor NORs Location
Coregoninae:					
Coregonus:					
C. clupeaformis	lake whitefish	2	A		
Salmoninae:					
Salvelinus:					
S. alpinus	arctic char	2	C'	0-6	A,B,C
S. confluentis	bull trout	2	A		
S. fontinalis	brook trout	2-4	A	2-6	A,B,C
S. leucomaenis	Iwana	2	A		
S. malma	Dolly Var. char	2	C'		
S. namaycush	lake trout	2-4	A,B	2-8	A,B,C,C'
Salmo:					
S. clarki	cutthroat trout	2	A'		
S. gairdneri	rainbow trout	2	A'		
S. salar	Atlantic salmon	2	A'		
S. trutta	brown trout	2	A		
Oncorhynchus:					
O. gorbuscha	pink salmon	2	D		
O. keta	chum salmon	2	B		
O. kisutch	coho salmon	2	A		
O. masu	masu salmon	2	A		
O. nerka	sockeye salmon	2	A'		
O. tshawytscha	chinook salmon	2	A		

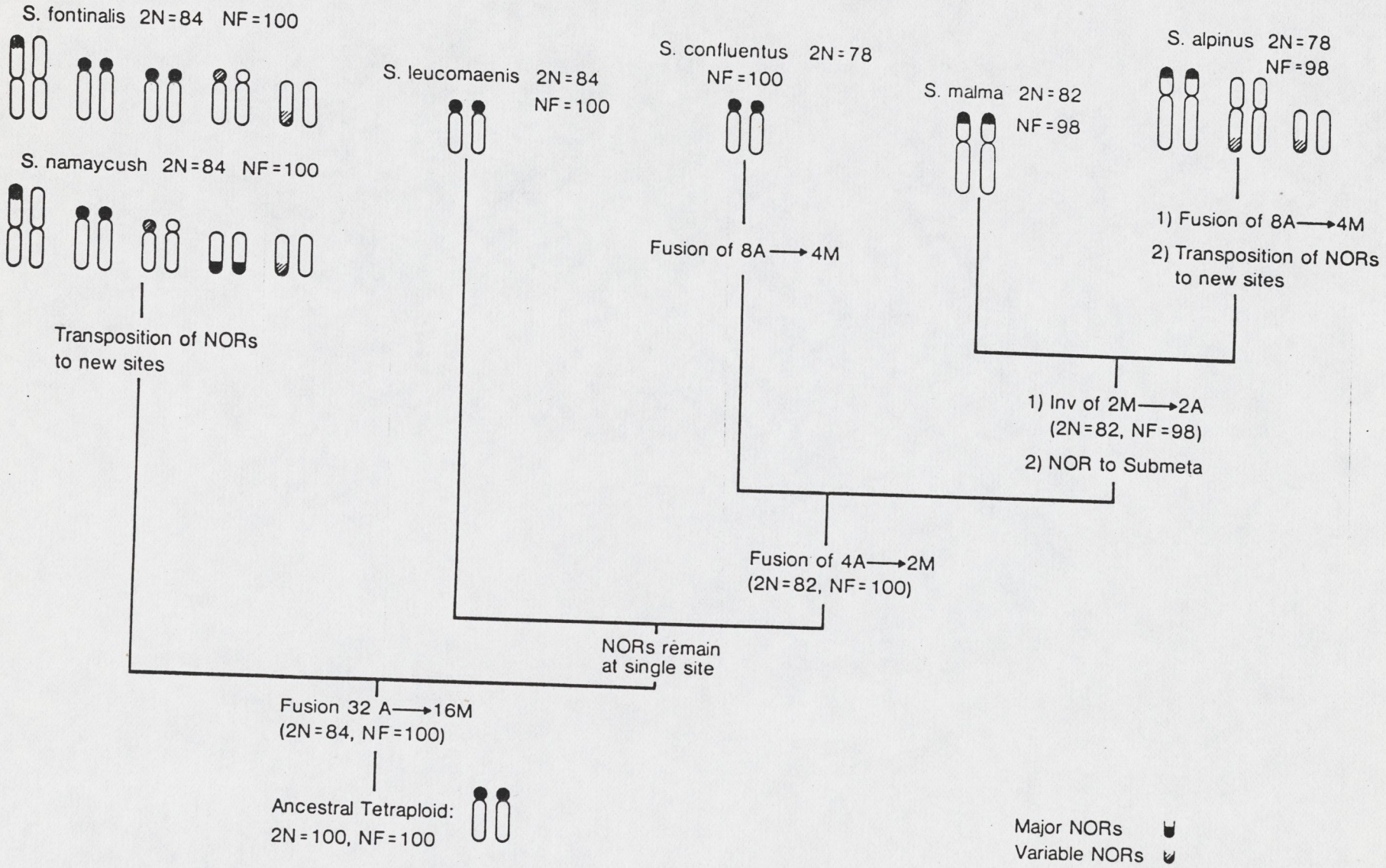
*A=acrocentric short arms, A'=submetacentric short arms, B=acrocentric telomeres, C=metacentric telomeres, C'=large submetacentric telomeres, D=metacentric adjacent to centromere

Figure Legends

Figure 1. Partial CMA3-stained karyotypes showing the location of NORs in the five Salvelinus species. In the species with multi-chromosomal location of NORs, intraspecific differences occur in the number and location of the variable NORs which are found on only one of the two homologous chromosomes, so that the partial karyotype shown is only one of several found for that species.

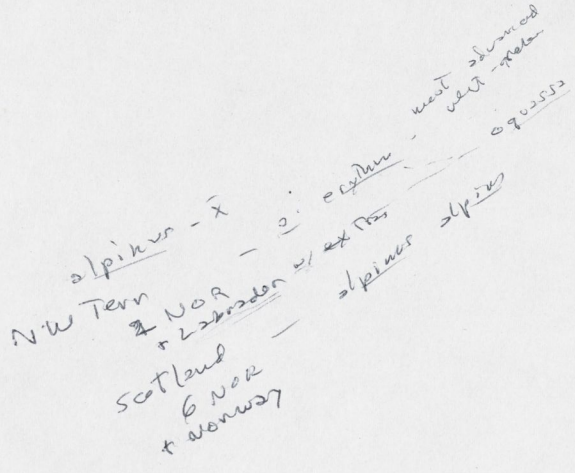
Figure 2. One possible cladogram showing relationships between Salvelinus species based on chromosome data. The locations of the major NORs which are always found on both members of a homologous pair in all members of the species are shown in black. The locations of the variable NORs which show intraspecific variation in number and chromosomal location and are usually found on only one member of a chromosome pair are shown in stippling. The chromosome data for all of the species except S. leucomaenis are based on karyotypes prepared from representatives of the five Salvelinus species from North America.

EVOLUTION OF NORs AND CHROMOSOMES IN THE GENUS SALVELINUS



Stock Differences in the Number and Chromosomal Position of NORs in arctic char (Salvelinus alpinus) --Phillips, Pleyte and Hartley, in press Cytogenetics and Cell Genetics

Abstract. Stock specific variation in the number and chromosomal location of the nucleolar organizer regions (NORs) was found in North American and European stocks of arctic char (Salvelinus alpinus) using chromomycin A3 (CMA3) staining and silver staining. The number of NORs varied from 2-8 per diploid genome. An unusual pattern of one constant NOR on both members of the largest submetacentric chromosome pair and additional variable NORs which were present on other chromosomes was found. These findings suggest that NORs are being transposed to new chromosomal sites in this species and this is discussed in relation to similar findings in other Salvelinus species and in amphibians.



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Stock differences in the chromosomal location of NORs in arctic char (Salvelinus alpinus). K. A. Pleyte and R. B. Phillips. University of Wisconsin-Milwaukee, Milwuakee, Wisconsin.

Previous work has shown that although most species of the ancestrally tetraploid subfamily Salmoninae (trouts and salmon) have only one NOR per haploid genome, several species of the genus Salvelinus are unique in having a multichromosomal location of NORs. (Phillips and Ihssen, Can. J. Genet. Cytol. 27:433-440, 1985). Stock differences in the number of NORs per genome and their chromosomal location have been found in lake trout (Salvelinus namaycush), with average numbers of NORs per diploid genome varying from 4 to 10. Most individuals have the NORs on both members of 1-3 chromosome pairs, but the additional NORs are usually found on only one homologous chromosome. Inheritance studies have shown that the NOR sites are inherited from the parents according to simple Mendelian principles. Similar findings have been reported for Triturus vulgaris, which has one major NOR site and additional single sites on one member of many chromosome pairs (Andronica et al, J. Mol. Biol. 186:219-229). Using silver staining and chromomycin A3/distamycin A staining, we examined the chromosomal location of the NORs in 8-20 different indviduals from 4 arctic char stocks from Northwest Territories, Labrador, Norway and Scotland. We found that the average number of NORs per diploid genome varied from 2 in the Northwest Territories to 6 in Scotland. All of the fish from the Northwest Territories have large NORs on both members of one homologous chromosome pair, the largest submetacentric in the karyotype. Many of these large NORs appear to contain double bands visible with both staining techniques. A few individuals have 1-3 additional NORs on single chromosomes. The Labrador fish have the same major NOR site, but there are additional sites on single chromosomes. The number of total NORs was greatest in the fish from Norway and Scotland, in which the NOR sites appear on 4-6 metacentric chromosomes and 2-3 acrocentric chromosomes in many individuals. Stock differences were also found in the number of bright Q bands per genome, which varied from zero in many fish from Northwest Territories to 15 in the Scottish fish.

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A Robertsonian polymorphism in pink salmon (*Oncorhynchus gorbuscha*) involving the nucleolar organizer region

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Abstract. A chromosome polymorphism involving a Robertsonian rearrangement has been found for two consecutive years in pink salmon from Lake Superior. Although the majority of the fish have a diploid number of 52, as previously reported for *Oncorhynchus gorbuscha*, 10–15% of the population have 2n = 53, in which one of the metacentric chromosomes has apparently undergone fission. The chromosome pair involved is the pair which contains the nucleolar organizer region (NOR) which is normally found adjacent to the centromere. In all of the individuals with 53 chromosomes, there is a small, acrocentric chromosome representing the short arm of the normal metacentric and a slightly larger, submetacentric chromosome with the NOR on its short arms. This chromosome apparently represents the long arm of the original metacentric in which the NOR has been rearranged to the other side of the centromere. Analysis of artificially induced haploid embryos as well as normal diploid embryos from females with 53 chromosomes suggests that meiotic chromosomes undergo alternate disjunction so that only balanced gametes are produced.

Most of the previous karyotypic studies of pink salmon from western North America and eastern Asia (Simon, 1963; Muramoto et al., 1974; Phillips et al., 1986) have found that all of the individuals studied had a diploid chromosome number of 52 with 24 pairs of metacentric or submetacentric chromosomes and two pairs of acrocentric or subtelocentric chromosomes. However, in one case (Gorshkov and Gorshkova, 1981), fish collected from the Kamchatka Peninsula were found to be polymorphic for chromosome number, with different individuals having 52, 53, or 54 chromosomes. The individuals with 53 or 54 chromosomes appeared to be either heterozygous or homozygous for a centric fission of the seventh largest metacentric chromosome pair.

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Request reprints from: Dr. R.B. Phillips, Department of Biological Sciences and Center for Great Lakes Studies. University of Wisconsin-Milwaukee, Milwaukee, WI 53201 (USA).

In this paper we report a similar but not identical chromosome number polymorphism in pink salmon from Lake Superior involving the chromosome pair with the nucleolar organizer region, which is the seventh largest metacentric chromosome pair (Phillips et al., 1986). This pair can be identified either with silver staining or with chromomycin A3 (CMA3) staining since CMA3 stains the nucleolar organizer regions in many fish and amphibian species (Schmid, 1982; Phillips and Ihssen, 1985). Previous work (Phillips et al., 1986) has shown that the structure of the nucleolar organizer region revealed with CMA3 staining is variable, with many individuals having two blocks of CMA3 staining material with an intervening negatively staining region on at least one homologue.

Materials and methods

Fertilized eggs of *Oncorhynchus gorbuscha* were obtained from the Minnesota Department of Natural Resources in the fall of 1984 and gametes were obtained from Lake Superior and fertilized in our laboratory in 1985. The parental fish were obtained from the Cross River in 1984 and from the Cross and French rivers in 1985.

Methods for inducing gynogenetic haploids are those of Thorgaard et al. (1983). Sperm is irradiated with UV light and the irradiated sperm is used to fertilize the eggs. A high percentage of haploids are obtained using this method. The haploids develop into embryos, but die shortly before hatching.

Chromosome slides were made from embryos dissected from fertilized eggs and incubated in culture media with 25 μg/ml colchicine as described previously (Thorgaard et al., 1981; Phillips and Zajicek, 1982). Preparations were made from the anterior kidney of fingerlings using the method of Kligerman and Bloom (1977). Slides were stained with silver nitrate using the method of Howell and Black (1980). For chromomycin A3 (CMA3) staining, 0.5 mg/ml was used and slides stained for 2 h in a moist chamber and then counterstained with distamycin A for 15 min. Slides were viewed with a Zeiss Universal microscope on bright field for the silver stained slides and with a B12 excitation filter and 47 or 50 barrier filter for the CMA3 stained slides. Photographs were made using Kodak Technical Pan film with standard development and 8 × 10 prints were made for karyotyping.

Results

About 10–15% of the individuals sampled in 1984 and 1985 had 53 chromosomes instead of 52, as has been found previously for all individuals of *O. gorbuscha* karyotyped from North America (Table I). In 1984, 24 embryos from a mass mating were tested and three of them had 53 chromosomes. In 1985, eggs from eight females were fertilized with sperm from eight males in different combinations, and haploid embryos were produced from each female by using irradiated sperm. Genotypes of the females were determined from chromosome constitutions of the haploids, and genotypes of the males were deduced from chromosome constitutions of the diploid progeny. One of the females and one of the males had 53 chromosomes.

All of the individuals with 53 chromosomes had the same karyotype. One member of the seventh-largest chromosome pair with the nucleolar organizer region (NOR) was missing and had been replaced by an additional submetacentric chromosome with an NOR and an acrocentric chromosome without an NOR. Partial karyotypes of the seventh-largest chromosome pair stained with CMA3 to reveal the NORs are shown in Fig. 1 from individuals with 52 or 53 chromosomes.

All of the individuals with 53 chromosomes had two CMA3-staining bands in the NORs on the metacentric and submetacentric chromosomes. Double CMA3 bands in the NOR have been found to be very common in pink salmon from Lake Superior (Phillips et al., 1986). Considerable variation occured in the size of the unstained region (Fig. 1). Silver staining of these chromosomes usually resulted in double bands, but in some cases there was a single, large band spanning part of both CMA3 bands and the intervening unstained region (Fig. 2). In all cases, sequential banding showed that the proximal CMA3 band extended right to the centromere, but the proximal silver band and the gap on Giemsa staining began a short distance below the centromere.

Inheritance of the extra chromosome was observed in haploid and diploid progeny of one female with 53 chromosomes (Table II). The results are consistent with a 1:1 ratio of individuals with 26 and 27 chromosomes in the haploid progeny. The sample size of

Table I. Frequency of *O. gorbuscha* individuals with different chromosome numbers

Location	Year	Life stage	Diploid number			Total
			52	53	54	
Seattle, WA	83	fingerlings	5			5
Cross River, MN	84	eggs	18	3		24[a]
Cross River, MN	85	adults	14	2		16
Kamchata, USSR	81	adults[b]				43

[a] Three of the 24 embryos examined were triploids.

[b] Seventy percent of 200 figures examined from 43 fish had 53 or 54 chromosomes; only eight had 52 (from Gorshkov and Gorshkova, 1981).

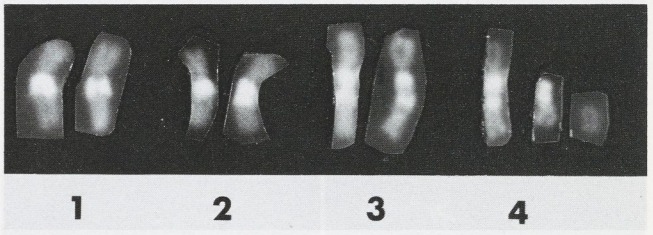

Fig. 1. Partial karyotypes of *Oncorhynchus gorbuscha* showing the chromosome pair with the NOR from four different individuals; stained with chromomycin A3 (CMA3). (1) An individual homozygous for single CMA3 bands, (2) an individual heterozygous for double CMA3 bands, (3) an individual homozygous for double CMA3 bands, and (4) an individual with 53 chromosomes: one metacentric with double bands, one acrocentric with double bands, and one small acrocentric without an NOR.

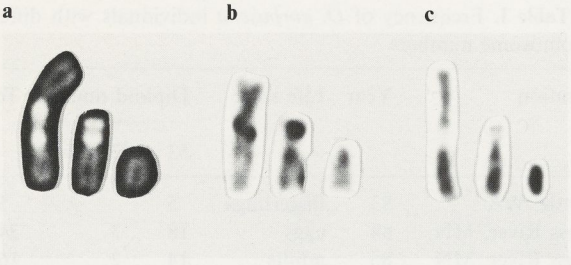

Fig. 2. Partial karyotypes of a female with 2n = 53 showing the three chromosomes homologous to the chromosome pair with NORs in fish with 2n = 52. These chromosomes are from karyotypes stained with: **(a)** CMA3, **(b)** silver nitrate, and **(c)** Giemsa.

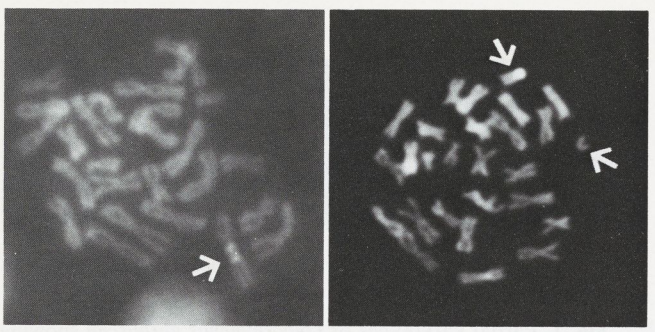

Fig. 3. Metaphase chromosomes from haploid individuals with **(a)** 26 chromosomes including a metacentric with an NOR, and **(b)** 27 chromosomes including two acrocentrics, one with an NOR, the other without an NOR.

Table II. Chromosome numbers in diploid and haploid progeny of a female *O. gorbuscha* with 53 chromosomes

Progeny	Chromosome number				Total	P^a
	26	27	52	53		
Haploid	11	16			27	.36
Diploid			7	3	10	–

[a] Probability from Chi-square test.

the diploid progeny was too small to allow any conclusions regarding segregation ratio, but all of the progeny had either 52 or 53 chromosomes. All of the diploid and haploid progeny observed appeared to be the result of alternate disjunction of chromosomes in meiosis, since they had either the metacentric chromosome with the NOR, or the small acrocentric and the small submetacentric with the NOR (Fig. 3).

Discussion

Robertsonian chromosome polymorphisms which may result from centric fusions or dissociations have been found in many species of salmonid fishes (reviewed in Gold, 1979, and Allendorf and Thorgaard, 1984). In most cases, it is not known whether these represent fusions or fissions, since individuals with various numbers of chromosomes are common in several populations. Recently Thorgaard et al. (1983) summarized chromosome data on 29 populations of rainbow trout with diploid numbers of 58 to 64. Since 2n = 58 is the most common number and is also found in the related red band and golden trout, he has proposed that the polymorphism in rainbow trout is the result of chromosome fission. In the present case, the majority of pink salmon which have been karyotyped had a chromosome number of 52, so that chromosome fission must have occurred to yield individuals with 53 chromosomes.

The only other case of a chromosome number polymorphism for pink salmon was reported for fish collected from the Kamchatka Peninsula by Gorshkov and Gorshkova (1981). These fish were spawned in 1979, an odd year. Even-year pink salmon on both sides of the north Pacific have been shown to be genetically more alike than odd- and even-year fish in the same locality since pink salmon normally have a rigid two-year life cycle (Aspinwall, 1974). The pink salmon in Lake Superior are the progeny of a single planting of fish in 1956 from an odd-year spawning the fall before (Kwain and Lawrie, 1981). Relatively few chromosome studies have been done on odd-year pink salmon from the west coast, so it is not known if chromosome number polymorphisms exist in these populations.

Although Gorshkov and Gorshkova (1981) analyzed only Giemsa-stained chromosomes, they reported that secondary constrictions adjacent to the centromere were sometimes seen in the metacentric and in the larger acrocentric of the chromosome pair involved in the fission. Since NORs often appear as secondary constrictions with Giemsa staining (Fig. 2), the chromosome pair involved in the fission was probably the same in the pink salmon studied from Kam-

chatka and Lake Superior. However the polymorphism found in the fish from Kamchatka resulted from a simple fission of one or two metacentric chromosomes. The majority of individuals had 53 chromosomes with one medium-sized acrocentric and one small acrocentric replacing one metacentric chromosome, and some individuals had 54 chromosomes with one metacentric chromosome pair being replaced by two medium-sized acrocentrics and two small acrocentrics. In the individuals with 53 chromosomes examined in the present study, a minimum of two events has occurred since the NOR is found on the short arm of a submetacentric (type 4, Fig. 1). The simplest explanation for the origin of this chromosome is that a centric fission was followed by inversion of the NOR to the other side of the centromere.

Double CMA3 and silver bands at the NOR have been reported in several amphibian populations (Schmid, 1982). Although Schmid reported that individuals were almost always heterozygous for the double-banded chromosomes, we have found that homozygotes are common in the pink salmon. Mayr et al. (1986) found that CMA3 stained primarily the flanking regions of the NOR in rainbow trout chromosomes which had a single large silver band, and similar results have been reported in *Hyla versicolor* (Wiley, personal communication), although there was some overlap between the CMA3 bands and silver bands. Our sequential banding results suggest that CMA3 stains adjacent heterochromatin in addition to part of the NOR in pink salmon chromosomes. The presence of double silver bands in the majority of chromosomes with double CMA3 bands suggests that the NOR is duplicated in these cases.

The results obtained from examination of haploid and diploid embryos from a female with 53 chromosomes suggested that disjunction is primarily alternate in heterozygotes resulting in production of gametes with either one metacentric chromosome with the NOR (26 chromosomes) or with the submetacentric with the NOR and the small acrocentric chromosome (27 chromosomes). These would result in balanced zygotes with 52 and 53 chromosomes when fertilized by a normal sperm. Although it is possible that aneuploid haploid embryos were produced but failed to survive, this seems unlikely for the diploid embryos since only one copy of each chromosome supports development in haploids until nearly the time of hatching. Normal disjunction in Robertsonian heterozygotes has been found in other species with fission polymorphisms (Porter and Sites, 1985). It is possible that selection favoring the gametes with 27 chromosomes may be occurring, but analysis of a larger number of haploid and diploid progeny from several heterozygotes would be needed to prove this. Unfortunately no crosses involving two fish with 53 chromosomes were performed, but these would be expected to result in fish with 54 chromosomes.

Future chromosome studies of the west coast founder population should be informative regarding whether the chromosome rearrangements occurred after planting in Lake Superior. We also plan to examine the frequency of the various genotypes in a larger sample from the Superior population in order to determine whether any of them are at a selective advantage in the new environment.

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Abstract:

Chromosome banding polymorphisms were examined in 8 different lake trout (Salvelinus namaycush) stocks from the Great Lakes region using quinacrine and CMA3 staining. Although considerable variation occurred between individuals in the number of bands with both staining techniques, the banding patterns were constant for all of the cells of a given individual. The stocks were differentiated into three groups on the basis of the number of CMA3 bands: a northern upper lakes group, a southern upper lakes group and a group comprising two stocks originally from Lake Michigan which are now in Wyoming. Each stock within these groups had a unique distribution of CMA3 bands on specific chromosomes, which was consistent when scored for two consecutive years. The stocks were very similar in the frequency of Q band variants, except for one polymorphism found in the Michipicoten Island, Ontario stock and another polymorphism which was rare in the Seneca Lake, New York stock. These same stocks were included in a genetic study of isozyme variation in Great Lakes lake trout stocks (Ihssen et al., 1988), and the largest electrophoretic genetic differentiation among the stocks included in this study was between the Seneca Lake, New York stock in the east and the other sympatric Great Lakes stocks. The sympatric stocks were similarly differentiated by the electrophoretic and cytogenetic data.

High Frequencies of Translocation Heterozygotes in Odd Year Populations of Pink Salmon (Oncorhynchus gorbuscha) --Phillips and Kapuscinski- submitted to Cytogen. and Cell Genet.

Abstract:

The pink salmon (Oncorhynchus gorbuscha) has a rigid two year life cycle, so that populations spawning on the even years do not exchange genes with populations spawning on the odd years. Examination of the chromosomes of two populations from an even year (1986) and four populations from an odd year (1987) showed that all individuals from the even year populations had a diploid number of 52, considered the normal number for the species, while a high frequency of individuals in each of the odd year populations sampled from Washington State to Alaska were translocation heterozygotes with a diploid chromosome number of 53. The chromosome involved in the translocation was the 7th metacentric pair containing the NOR (nucleolus organizer region) adjacent to the centromere. In two populations a simple fission of this chromosome has produced individuals with 53 chromosomes with two acrocentrics replacing the metacentric chromosome, with the larger acrocentric having the NOR adjacent to the centromere on the long arm. In the other two populations individuals with 53 and 54 chromosomes were found in which the acrocentric with the NOR has undergone an inversion so that the NOR is now on the short arm of a small submetacentric chromosome. In one population all of the individuals with 53 and 54 chromosomes were of this type, while in the other case both forms were found. Because these two populations are adjacent to each other in the middle of the range sampled, the rearranged chromosome probably had a single origin.

Chromosome banding in salmonid fishes: nucleolar organizer regions in *Oncorhynchus*

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PHILLIPS, R. B., K. D. ZAJICEK, and F. M. UTTER. 1986. Chromosome banding in salmonid fishes: nucleolar organizer regions in *Oncorhynchus*. Can. J. Genet. Cytol. **28**: 502–510.

Chromosome banding patterns obtained by silver staining and chromomycin a3 (CMA3) staining were analyzed in six species of *Oncorhynchus*: *O. tshawytscha*, *O. kisutch*, *O. keta*, *O. nerka*, and *O. gorbuscha* from North America and *O. masou* from Japan. Four different chromosomal locations of the nucleolor organizer regions (NORs) were found in different species. In *O. tshawytscha*, *O. kisutch*, and *O. masou* the NORs comprised the entire short arms of one medium-sized acrocentric chromosome pair. In *O. nerka* the NORs were found in an interstitial band on the short arms of one submetacentric chromosome pair and in *O. gorbuscha* proximal to the centromere on one metacentric chromosome pair. In *O. keta* the NORs were found on the telomeres of one small submetacentric chromosome pair. As in the related genera *Salmo* and *Salvelinus* chromomycin A3 positive bands were found at the same sites as the AgNORs in all species. Salmonid fish are assumed to be ancestral tetraploids and the considerable differences in chromosome number between different species are thought to be the result of chromosomal fusions after tetraploidization. In all members of the genus *Oncorhynchus* the rearrangements have resulted in the consolidation of the NORs on a single chromosome pair. The possible significance of intra- and inter-species NOR polymorphisms is discussed.

Key words: nucleolar organizer regions, salmon, *Oncorhynchus*, chromosomes.

PHILLIPS, R. B., K. D. ZAJICEK et F. M. UTTER. 1986. Chromosome banding in salmonid fishes: nucleolar organizer regions in *Oncorhynchus*. Can. J. Genet. Cytol. **28**: 502–510.

Les patterns de bandes chromosomiques par coloration à l'argent (Ag-NORs) et coloration à la chromomycine A3 (CMA3) ont été analysés pour six espèces d'*Oncorhyncus*: *O. tshawytscha*, *O. kisutch*, *O. keta*, *O. nerka* et *O. gorbuscha* de l'Amérique du Nord et *O. masou* du Japon. Quatre sites chromosomiques NORs différents ont été trouvés chez différentes espèces. Chez *O. tshawytscha*, *O. kisutch* et *O. masou*, les NORs couvraient les bras courts entiers de la paire de chromosomes acrocentriques de grandeur moyenne. Chez *O. nerka*, les NORs furent trouvés dans une bande interstitielle sur les bras courts d'une paire de chromosomes submétacentriques et, chez *O. gorbuscha*, les NORs étaient à proximité du centromère chez une pairde de chromosomes métacentriques. Chez *O. keta*, les NORs furent trouvés sur les télomères d'une petite paire de chromosomes submétacentriques. Tout comme pour les genres apparentés *Salmo* et *Salvelinus*, les bandes positives à la chromomycine A3 ont été trouvées aux mêmes sites que les Ag-NORs chez toutes les espèces. Les poissons salmonidés sont présumés être les ancêtres tétraploïdes et les différences considérables dans le nombre de chromosomes entre les différentes espèces sont considérées comme le résultat de fusions de chromosomes après la tétraploïdisation. Chez tous les membres du genre *Oncorhyncus*, les réarrangements ont conduit à la consolidation des NORs sur une seule paire de chromosomes. La signification possible de polymorphismes intra- et inter-espèces est discutée.

Mots clés: NORs, saumon, *Onchorhynchus*, chromosomes.

[Traduit par la revue]

Introduction

The silver staining of the nucleolar organizer regions (NORs) is the simplest method of demonstrating the position of the 18S and 28S ribosomal RNA cistrons in chromosomes (reviewed in Howell 1982) and has been applied to many vertebrate and invertebrate species. This technique stains only the NORs that have been active at the previous interphase (Howell 1977), so that differences in the number of NORs per cell may reflect differences in the number of active NORs. Each species appears to have a characteristic maximum number of chromosome pairs with NORs, although polymorphisms in the relative size of the NORs on homologous chromosomes and the number of active NOR sites per cell have been found for most species examined (Howell 1982).

In anuran amphibians it has recently been shown (Schmid 1982) that the AgNOR regions stain brightly with chromomycin A3 (CMA3), possibly because of the GC-rich spacer region in the rDNA. CMA3 stains the rDNA, regardless of its activity, so that with CMA3 staining it was possible to identify the inactive NORs in

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TABLE 1. Chromosomal location of NORs in different species of *Oncorhynchus*

Scientific name	Common name	No. of NOR chromosome pairs	Specific NOR location	2*n*
O. tshawytscha	Chinook salmon	1	Acrocentric, short arms	68
O. kisutch	Coho salmon	1	Acrocentric, short arms	60
O. masou	Masu salmon	1	Acrocentric, short arms	68
O. keta	Chum salmon	1	Submetacentric, telomeres	74
O. nerka	Sockeye salmon	1	Submetacentric, interstitial	56
O. gorbuscha	Pink salmon	1	Metacentric, interstitial	52

various stages of anuran spermatogenesis, in which there is no rRNA synthesis. In previous work on salmonid fishes, we found that CMA3 stained the AgNORs in six species of *Salmo* and *Salvelinus* (Phillips 1983; Phillips and Ihssen 1985*a*).

Only one chromosome pair with NORs has been found among fishes of diploid ancestry (Howell and Black 1980; Kornfield et al. 1979; Kligerman and Bloom 1977; Foresti et al. 1981; Takai and Ojima 1982) with the exception of one cyprinid species (Gold 1984). However, salmonid fishes appear to be tetraploids in the process of diploidization (reviewed by Gold 1979; Allendorf and Thorgaard 1984), so that they might be expected to have more than one chromosome pair with NORs.

Previous studies on the NOR regions in various species of the genera *Salmo* and *Salvelinus* (Phillips 1983) have shown that while most species of *Salmo* have only one chromosome pair with NORs, two members of the genus *Salvelinus* have NORs on at least four to six chromosome pairs. Different salmonid species have apparently undergone different numbers of chromosomal fusions involving about 100 chromosome arms since the 2*n* chromosome number varies from 52 to 84. Consequently the chromosomal locations of the NORs in the different species might yield useful information regarding these chromosomal changes and the relationships between the various species.

The present paper reports the chromosomal location and polymorphisms of the nucleolar organizer regions as revealed by silver staining and CMA3 staining in individuals from six species of the genus *Oncorhynchus*. The purpose of the study is to provide a basis for more extensive intraspecific studies and to make initial interspecific comparisons.

Materials and methods

Fertilized eggs and fingerlings of *O. tshawytscha* (chinook salmon) and *O. kisutch* (coho salmon) were obtained from the fish hatcheries of the Wisconsin Department of Natural Resources, and fertilized eggs of *O. gorbuscha* (pink salmon) were obtained from the Minnesota Department of Natural Resources. Fertilized eggs of *O. keta* (chum salmon) and *O. tshawytscha* (chinook salmon) and fingerlings of *O. gorbuscha* (pink salmon), *O. nerka* (sockeye salmon), and *O. masou* (masu salmon) were obtained from the National Marine Fisheries Service, Seattle, Washington.

Chromosome preparations were made from two age-classes. Embryos were dissected from fertilized eggs and incubated in culture media with 25 μg/mL colchicine as described previously by Thorgaard et al. (1981) and Phillips and Zajicek (1982). Fingerling preparations were made from the anterior kidney using the method of Kligerman and Bloom (1977). Slides were stained with silver nitrate using the modifications of the Goodpasture and Bloom (1975) method described by Howell and Black (1980) and Gold (1984). For chromomycin A3 staining, 0.5 mg/mL was used and slides were stained for 1–2 h in a moist chamber and then counter stained with distamycin A for 10–15 min. Slides were viewed with a Zeiss Universal microscope on bright field for the silver-stained slides and with a B12 excitation filter and 47 or 50 barrier filter for the CMA3-stained slides. Photographs were made using Kodak Technical Pan film with standard development and 8 × 10 prints were made for karyotyping.

Results

Chromosomal location of the Ag-NORs

Twenty-four embryos and six fingerlings of *O. tshawytscha* were examined for nucleolar organizer chromosomes. In all cases the NORs were found to comprise the entire short arms of an acrocentric chromosome pair (described as one of the two small submetacentric chromosome pairs in previous karyotype descriptions (Simon 1963; Phillips et al. 1985)) (see Table 1, Figs. 1

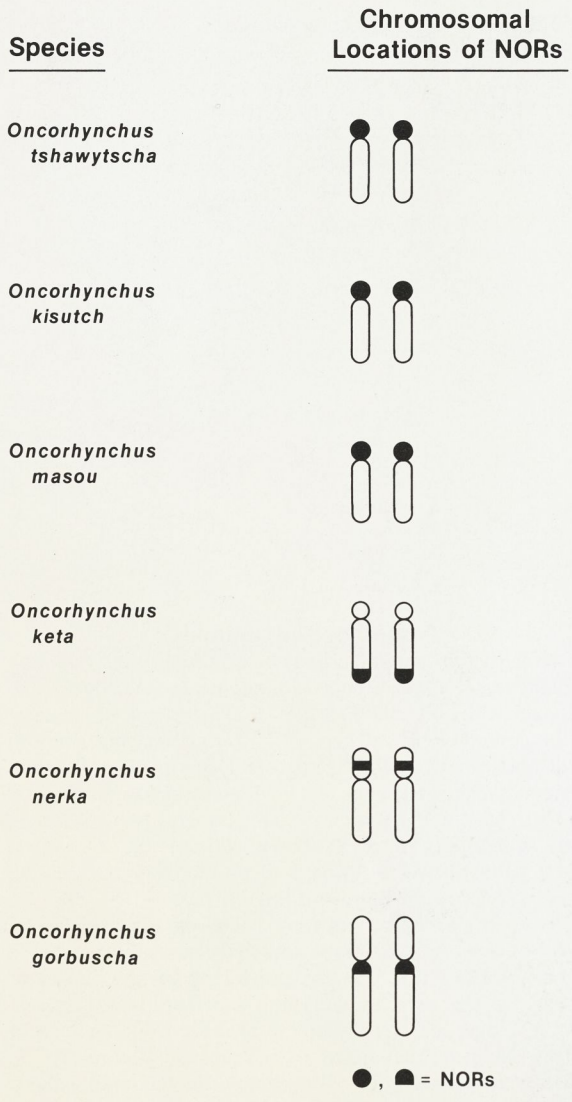

FIG. 1. Diagram of the chromosomes with nucleolar organizer regions in different species of *Oncorhynchus*.

and 2*a*). All individuals had $2n = 68$ chromosomes, Interphase cells had either one or two nucleoli.

Ten embryos and four fingerlings of *O. kisutch* were examined for nucleolar organizer chromosomes. In all cases the NORs were found to comprise the entire short arms of an acrocentric chromosome pair (see Table 1, Figs. 1 and 2*b*) and all fish had $2n = 60$ chromosomes. Interphase cells had only one or two nucleoli as in *O. tshawytscha*.

Four fingerlings of *O. masou* were examined for nucleolar organizer chromosomes. The karyotypes of these fish were very similar to those of *O. tshawytscha* with 66 chromosomes and one acrocentric nucleolar organizer chromosome pair (see Table 1, Figs. 1 and 2*c*). Interphase cells had one or two nucleoli.

Four fingerlings of *O. nerka* were examined for nucleolar organizer chromosomes. In all cases NORs were found on one submetacentric chromosome pair (No. 22 in size) in the middle of the short arm, corresponding to the secondary constriction found by previous workers (Thorgaard 1978; Saski et al. 1968). Interphase cells had either one or two nucleoli.

Twenty-four embryos of *O. keta* were examined for nucleolar organizer chromosomes. The NORs were found at the telomeres of one of the three small submetacentric chromosome pairs (Table 1, Figs. 1 and 3*a*). Cells had one or two nucleoli. This was the only *Oncorhynchus* species that had telomeric NORs.

Twenty-four embryos of *O. gorbuscha* from Minnesota and six fingerlings from Washington state were examined for nucleolar organizer chromosomes. The NORs were found next to the centromere of one metacentric chromosome pair in most individuals (Table 1, Figs. 1 and 5). One of six fingerlings and 3 of 24 embryos examined were triploids and these individuals had three nucleolar organizer chromosomes as would be expected for triploid cells.

In the *O. gorbuscha* embryos, double NOR regions were found in over half of the individuals examined, with many being heterozygous for this variant (see Table 2, Figs. 4 and 5). In addition three exceptional cases were found in which one of the two chromosomes with NORs was an acrocentric chromosome ending in a double NOR, with the rest of the short arm missing. These individuals had an additional small telocentric chromosome that was the same size as the missing arm, giving them a total chromosome number of 53 instead of the normal 52 (see Figs. 4 and 5*c*). The frequencies of these different types found among 24 embryos examined are given in Table 2.

CMA3 staining of NORs

In all of the species examined with both staining techniques except two, there appeared to be an exact correspondence between regions stained with silver and those stained with CMA3 counterstained with distamycin A. Sequential staining was done on a few individuals and in each of these cases, the chromosomes were shown to be identical. In *O. tshawytscha*, *O. kisutch*, and *O. masou*, CMA3 stained the short arms of an acrocentric pair of the same size as the one whose short arms stained with silver, and no other CMA3 positive regions were found in the karyotype. No chromosome preparations adequate for CMA3 staining were available for *O. nerka*. In all 24 of the individuals of *O. gorbuscha* examined, the same sites that were positive with silver staining were also positive with CMA3 but in addition centromeres of one chromosome pair were CMA3 positive in some individuals.

Oncorhynchus keta was exceptional in that CMA3 stained not only the AgNOR regions but also the

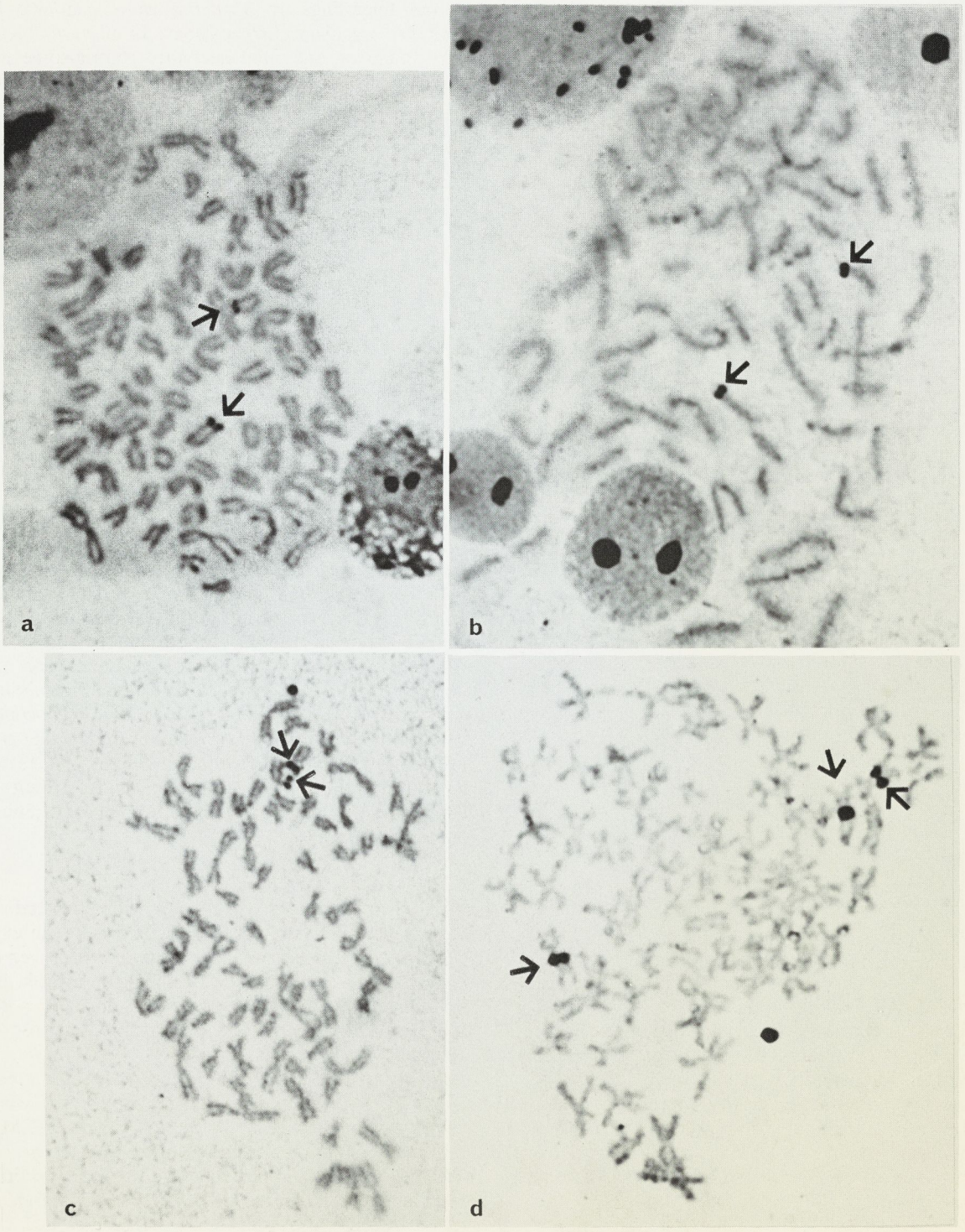

FIG. 2. Silver-stained metaphases of (*a*) chinook salmon, (*b*) coho salmon, (*c*) masu salmon, and (*d*) pink salmon. Silver-stained nucleolar organizer regions are indicated by arrows. In Fig. 2*d*, the pink salmon is a triploid with three NORs. See Fig. 5 for diploid pink salmon.

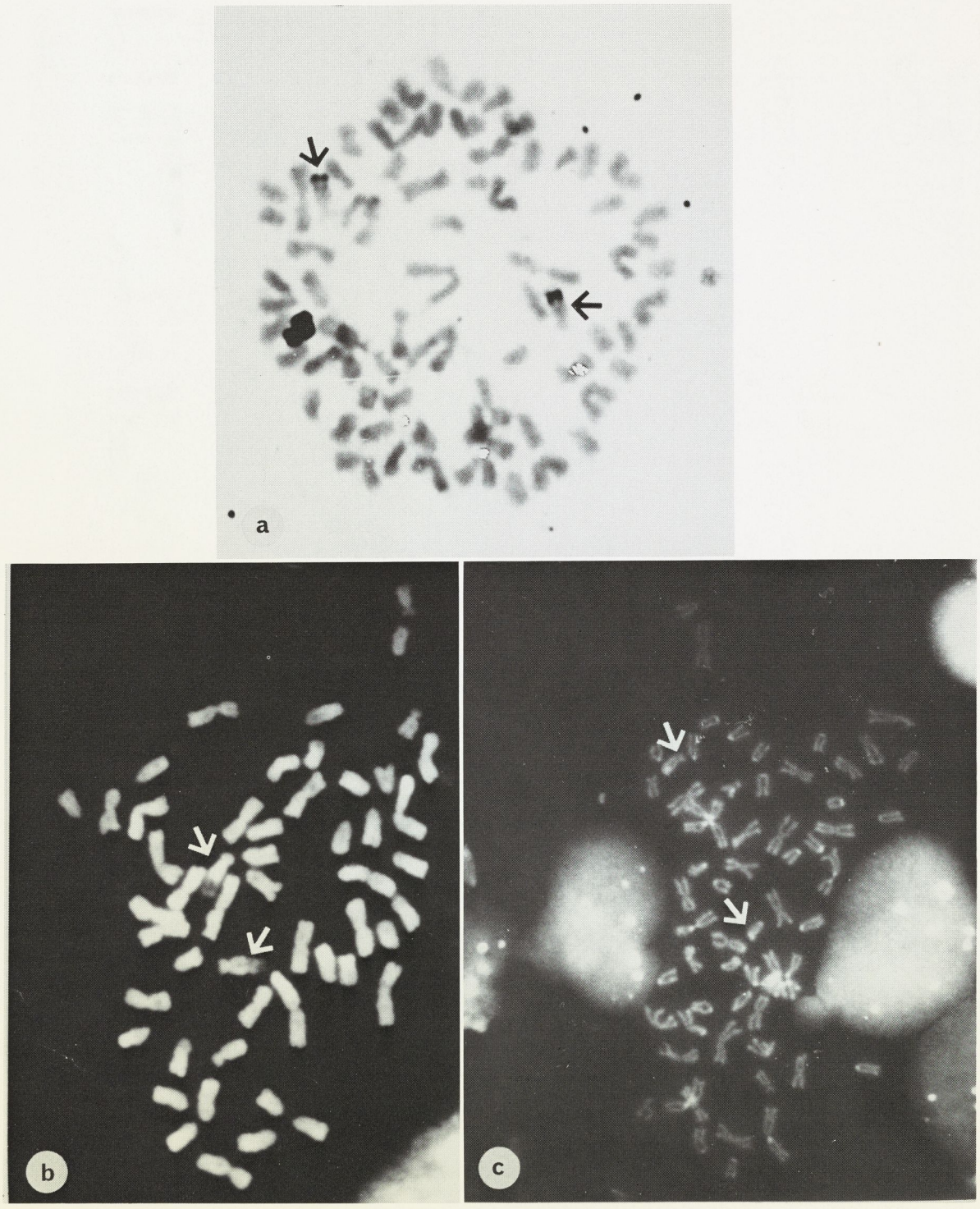

FIG. 3. Metaphases of chum salmon stained with (*a*) silver staining, (*b*) quinacrine, and (*c*) chromomycin A3 counterstained with distamycin A. Arrows indicate the nucleolar organizer regions on a small metacentric chromosome pair.

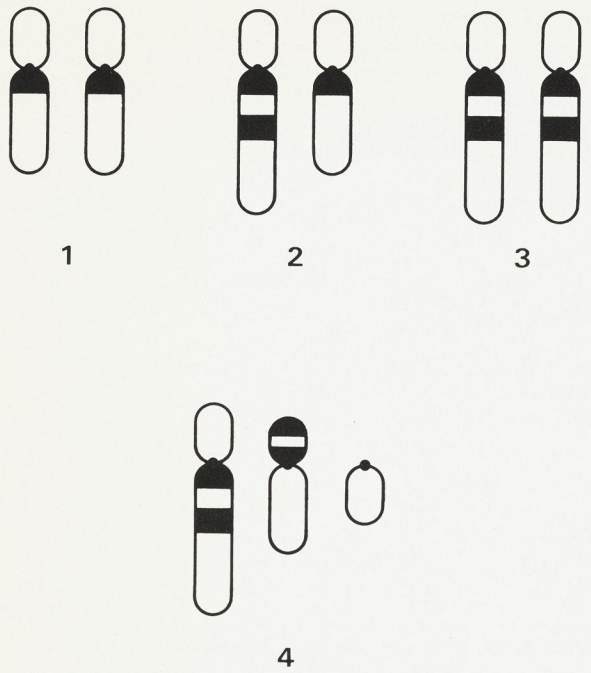

FIG. 4. Diagram of the different types of chromosomes found with nucleolar organizer regions in different diploid pink salmon individuals. Black regions are those which stained with silver and chromomycin A3. In type 4, one member of the pair of metacentric chromosomes has apparently split into two smaller chromosomes, so that these individuals have 53 chromosomes. One of these smaller chromosomes has the duplicated NOR on the short arm and the other chromosome is a small telocentric lacking an NOR.

centromeres of many other chromosome pairs including all of the telocentric chromosomes (see Fig. 3*c*). Thus it appears that the centromeric heterochromatin on the telocentric chromosome pairs and on one metacentric chromosome pair in this species may have GC-rich sequences. The NOR regions were negatively staining with quinacrine, while the centromeres were variable with Q-banding (see Fig. 3*b*).

The double NORs in *O. gorbuscha* were especially obvious with CMA3 staining. Chromosomes from individuals of types 1, 2, and 4 from Table 2 and Fig. 4 that were stained with CMA3 are illustrated in Figs. 5*a*, 5*b*, and 5*c*. Two distinct bands at the truncated end of the derived chromosome in individuals of type 4 can be seen with CMA3 staining (Fig. 5*c*).

Discussion

The North American *Oncorhynchus* species can be divided into three groups on the basis of chromosomal location of NORs. The NORs are on short arms of one acrocentric pair in *O. tshawytscha* and *O. kisutch* and

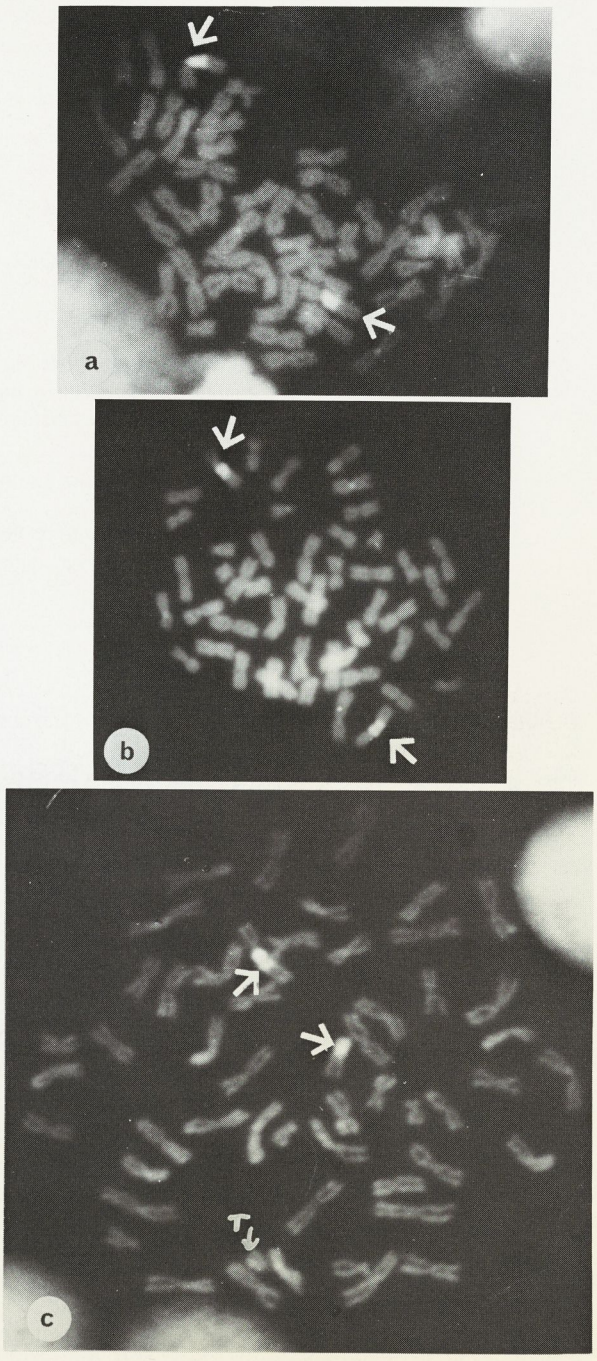

FIG. 5. Metaphases from three different individuals of pink salmon stained with chromomycin A3. Arrows indicate the nucleolar organizer regions. These correspond to the types shown in Fig. 4: (*a*) type 1, (*b*) type 2, and (*c*) type 4. The small telocentric in type 4 is indicated by a small arrow with the letter T.

TABLE 2. Frequencies of NOR polymorphisms in *O. gorbuscha**

Description of genotype	No. observed	
	Embryos	Fingerlings
Diploids		
Type 1: two chromosomes with NORs, each with a single band	2	5
Type 2: two chromosomes with NORs, one with a single band, one with a double band	13	—
Type 3: two chromosomes with NORs, each with a double band	4	—
Type 4: two chromosomes with NORs, one metacentric, the other acrocentric, both with a small telocentric present as a 53d chromosome	3	—
Triploids		
Type 1: three chromosomes with NORs, all with single bands	—	1
Type 2: three chromosomes with NORs, two with double bands, one with a single band	2	—
Total	24	6

*Embryos were from Lake Superior and fingerlings from Seattle, Washington. The different diploid genotypes are illustrated in Fig. 4.

near the centromere on metacentrics or submetacentrics in *O. gorbuscha* and *O. nerka*. In *O. keta* the NORs are found at the telomeres of a small submetacentric chromosome. These groups correspond well to those obtained from biochemical, morphological, and ecological data (reviewed in Benke 1965 and Utter et al. 1973).

The Asian *O. masou* has been considered to be the most primitive living *Oncorhynchus* species with considerable affinities to *Salmo gairdneri* on the basis of morphological and biochemical data (Benke 1965; Utter et al. 1973). Examination of the karyotype of *O. masou* reveals that it is very similar to that of *O. tshawytscha* (Muramoto et al. 1974). *Oncorhynchus tschawytscha* has a diploid number of 68, with 32 meta- and submeta-centric chromosomes and 36 acrocentric and telocentric chromosomes, while *O. masou* has a diploid number of 66, with 34 meta- and submeta-centric chromosomes and 32 acrocentric and telocentric chromosomes. The NORs are found on the short arms of an acrocentric chromosome of similar size in each karyotype. Although G-banding will be required to prove homology of these chromosomes and construct an accurate phylogenetic tree for these species, the available karyotypic data suggests that *O. masou* may be more closely related to *O. tschawytscha* than any of the other North American species.

It has been shown recently that CMA3 stains the NOR region regardless of activity in anuran amphibians (Schmid 1982), and Monaco et al. (1981) reported that CMA3 stained the multiple nucleoli in oocytes of *Salmo gairdneri*. Our results in *Salmo*, *Salvelinus* (Phillips and Ihssen 1985*a*), and *Oncorhynchus* suggest that CMA3 stains the NOR regions regardless of activity in all members of the Salmoninae.

In some amphibians, CMA3 stained certain heterochromatin sequences in addition to the NOR (Schmid 1982) and that appears to be the case in *O. keta* and *O. gorbuscha*. In all other *Oncorhynchus* species there was an exact correspondence between CMA3-stained and AgNOR-stained regions. *Oncorhynchus keta* is also the only species with NORs at the telomeric chromosome region. These unique karyotypic features corroborate the biochemical evidence (Utter et al. 1973) that this species is not closely related to any of the others.

The number of nucleoli per cell varied from one to two in all of the *Oncorhynchus* species examined, with cells of both types found in every individual. Many of the cells with one nucleolus probably result from the two NOR-bearing homologous chromosomes lying next to each other, since no fish were found in which all cells had only one chromosome with NORs. The triploid individuals found in *O. gorbuscha* had three chromosomes with NORs and the majority of their cells had three nucleoli. Recent experiments have shown that counting nucleoli in silver-stained preparations is a simple method of identification of triploid cells in several species of *Oncorhynchus* and *Salmo* (Phillips et al. 1986).

Salmonids are thought to be tetraploids in the process of diploidization (reviewed in Gold 1979); Allendorf and Thorgaard 1984). In *Oncorhynchus* and *Salmo*, it appears that diploidization has resulted in the consolida-

tion of the NOR region onto one chromosome pair in all of the species, while dispersion onto additional pairs has occurred in the case of two *Salvelinus* species.

Intraspecific polymorphisms in the nucleolar regions have been found in many species, including the relatively few fish that have been examined for NORs (Foresti et al. 1981; Gold 1984). These polymorphisms include differences in the relative size of the NOR region in homologous chromosomes, duplication of NORs, and variations in the number of active NOR sites per cell. Differences in the presence and staining properties of adjacent heterochromatin have also been found. These polymorphisms have been shown to be heritable in humans (Phillips 1975; Taylor and Martin-DeLeon 1981). In species with multiple NORs, it is especially common to find considerable variation in the number of active NOR sites per cell with most cells having only 70–80% of the NORs active.

In salmonid fishes of the genus *Salvelinus*, we have found all of these types of polymorphisms (Phillips and Ihssen 1985*a*). In lake trout, which have NORs on four to six chromosome pairs, we have found population differences in the total number of NORs per cell as determined by CMA3 staining as well as in the chromosomal distribution of NORs. Stocks from Jenny Lake, Wyoming, had an average of 3.9 NORs per cell compared with 8.9 NORs per cell for stocks from Lake Superior (Phillips and Ihssen 1985*b*).

In salmonid fishes of the genus *Oncorhynchus*, all of the species showed intraspecific variations in the size of the NORs on homologous chromosomes, but intraspecific polymorphisms involving duplicated NORs or variations in chromosomal location of NORs were found only in *O. gorbuscha*. The variation in chromosome location of the NOR found in a few individuals appeared to be the result of a chromosome fission at the centromere of the NOR-bearing chromosome, which was followed by rearrangement, since these individuals had 53 chromosomes.

In anurans of several species collected from the wild, individuals heterozygous for double NORs similar to those found in *O. gorbuscha* were found at a frequency of 60%, but no individuals homozygous for double NORs were found (Schmid 1982). In *O. gorbuscha* embryos we found some individuals homozygous for double NORs. Variations between homologous chromosomes were found in the size of the space between the two CMA3 bands, which is reduced in size in highly condensed chromosomes. Further studies are needed to determine the functional significance of the double NORs in *O. gorbuscha*.

Double NORs and karyotypes with rearranged NORs and chromosomes were found only in *O. gorbuscha* embryos that were collected from Lake Superior. These fish are the descendants of a single planting of Skeena River, B.C., fish derived from eggs fertilized in 1955, an odd year. The six fingerlings obtained from Washington State were also from an odd year spawning. However, electrophoretic studies have shown that odd year Puget Sound pink salmon are genetically distinct from odd year populations found further north (Beacham et al. 1985). Differences in isozyme frequencies are greatest between odd and even year pink salmon that do not interbreed in their native habitat (Aspinwall 1974), but chromosomal polymorphisms have not been examined in these broodlines. Future studies of a larger number of individuals are needed to determine whether the chromosomal polymorphisms identified in this study will be useful as genetic markers in populations of *O. gorbuscha*.

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Chromosome banding in salmonid fish: nucleolar organizer regions in *Salmo* and *Salvelinus*

R. Phillips and P. E. Ihssen

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Chromosome banding in salmonid fish: nucleolar organizer regions in *Salmo* and *Salvelinus*

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PHILLIPS, R., and P. E. IHSSEN. 1985. Chromosome banding in salmonid fish: nucleolar organizer regions in *Salmo* and *Salvelinus*. Can. J. Genet. Cytol. **27**: 433–440.

Chromosome banding patterns obtained by silver staining (Ag-NORs) were analyzed in three species of *Salmo* (rainbow, brown trout, and Atlantic salmon) and three species of *Salvelinus* (brook trout, lake trout, and arctic char). In rainbow trout and Atlantic salmon the Ag-NORs were found at the secondary constrictions of a single chromosome pair, while in brown trout the Ag-NORs were found on the short arms of one or two of the two longest subtelocentric or acrocentric chromosome pairs. The location of the Ag-NORs was multichromosomal in the three *Salvelinus* species, occurring on one or both members of four to six different chromosome pairs in different individuals. The Ag-NOR sites were on the short arms of some acrocentric pairs and at the telomeres of other acrocentric pairs and one or two metacentric pairs. Chromomycin A3 positive bands were found at the same sites as the Ag-NORs in all species. In the species with multichromosomal location of Ag-NORs, polymorphisms in the size and location of the NORs were extremely common, so that almost every individual fish had a different pattern of Ag-NOR sites.

Key words: banding, *Salmo*, *Salvelinus*, Ag-NORs, polymorphisms, nucleolar organizer.

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Des patterns de bandes chromosomiques ont été obtenus par coloration à l'argent (Ag-NORs) et analysés chez trois espèces de *Salmo* (la truite arc-en-ciel, la truite brune et le saumon de l'Atlantique) et chez trois espèces de *Salvelinus* (la truite des ruisseaux, la truite des lacs et l'ombre de l'arctique). Chez la truite arc-en-ciel et le saumon de l'Atlantique, les Ag-NORs furent trouvés dans les constrictions secondaires d'une seule paire de chromosomes, alors que chez la truite brune les Ag-NORs furent trouvés sur les bras courts d'une ou de deux paires de chromosomes acrocentriques ou subtélocentriques les plus longues. La localisation des Ag-NORs fut multi-chromosomique chez les trois espèces de *Salvelinus*, survenant sur un ou sur les deux membres de quatre des six paires de chromosomes différents chez différents individus. Les sites d'Ag-NOR furent localisés sur les bras courts de certaines paires d'acrocentriques, sur les télomères d'autres paires acrocentriques et sur une ou deux paires métacentriques. Des bandes positives à la chromomycine A3 ont été trouvées aux mêmes sites que le Ag-NORs chez toutes les espèces. Chez les espèces à localisation multi-chromosomique d'Ag-NORs, le polymorphisme fut des plus communs quant aux localisations et dimensions des Ag-NORs, de sorte que presque chaque poisson avait un pattern différent des sites d'Ag-NOR.

Mots clés: bandes chromosomiques, *Salmo, Salvelinus*, Ag-NORs, polymorphisme, organisateur nucléolaire.
[Traduit par le journal]

Introduction

The silver (Ag) staining of the nucleolus organizer regions (NORs) is the simplest method of demonstrating the position of the genes for 18s and 28s ribosomal RNA in chromosomes (reviewed in Howell 1982) and has been applied to many vertebrate and invertebrate species. This technique stains only the NORs which have been active at the previous interphase (Howell 1977), and size polymorphisms on homologous chromosomes have been found for most species examined. In anuran amphibians it has recently been shown (Schmid 1982) that the Ag-NOR regions stain brightly with chromomycin A3, possibly because of the GC-rich spacer region in the rDNA. Chromomycin A3 stains the rDNA, regardless of its activity, so that with the chromomycin A3 staining it was possible to identify inactive NORs in stages of spermatogenesis, in which there is no rRNA synthesis.

Previous studies on NOR regions in fishes have revealed only one NOR-bearing chromosome pair in

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TABLE 1. Chromosomal location of NOR regions in different species of salmonid fish

Scientific name	Common name	No. of NOR chromosome pairs	Type of NOR chromosome	$2n$
Salmo gairdneri	Rainbow trout	1	Metacentric	60
Salmo salar	Atlantic salmon	1	Metacentric	56
Salmo trutta	Brown trout	2	Acrocentrics	80
Salvelinus fontinalis	Brook trout	4–6	Metacentrics, acrocentrics	84
Salvelinus namaycush	Lake trout	4–6	Metacentrics, acrocentrics	84
Salvelinus alpinus	Arctic char	2–4	Metacentrics, acrocentrics	82

the species studied (Howell and Black 1980; Kornfield et al. 1979; Kligerman and Bloom 1977; Foresti et al. 1981; Takai and Ojima 1982). However, most of these species have a chromosome number of approximately $2n = 50$. Salmonid fish are thought to be tetraploids in the process of diploidization since they contain approximately twice the DNA of related species (reviewed by Gold 1979) and the number of chromosome arms in many species is about 100. Different species have apparently undergone different numbers of chromosomal fusions since the $2n$ chromosome number varies from 52 to 84. The location of the NOR-bearing chromosome pairs in the different salmonid species might yield useful information regarding these chromosomal changes. The present paper reports the chromosomal location and polymorphisms of the NOR regions as revealed by silver staining and chromomycin A3 staining in six species of the genera *Salmo* and *Salvelinus*.

Materials and methods

Fertilized eggs of rainbow trout and brown trout and fertilized eggs and fingerlings of brook trout and lake trout were obtained from fish hatcheries of the Wisconsin Department of Natural Resources in the fall of 1982. Chromosome slides of adult arctic char and brook trout were obtained from James E. Wright, Jr., of Pennsylvania State University. Fingerlings of Atlantic salmon were obtained from the Ontario Ministry of Natural Resources.

Chromosome slides were made from embryos dissected from fertilized eggs and incubated in culture media with 25 μg/mL colchicine as described previously (Thorgaard et al. 1981; Phillips and Zajicek 1982). Preparations were made from the anterior kidney of fingerlings using the method of Kligerman and Bloom (1977). Slides were stained with silver nitrate using the method of Howell and Black (1980). For chromomycin A3 staining, 0.5 mg/mL was used and slides were stained for 1–2 h in a moist chamber and then counterstained with distamycin A for 10–15 min. For sequential staining, slides were washed in water after removal of the cover slip and dried before silver staining. Slides were viewed with a Zeiss universal microscope on bright field for the silver-stained slides and with a BG 12 excitation filter and 47 or 50 barrier filter for the fluorescent-stained slides. Photographs were made using Kodak Technical Pan film with standard development, and 8 × 10 prints were made for karyotyping.

Results

Chromosomal location of the Ag-NORs

Rainbow trout

All of the rainbow trout examined had only one NOR-bearing chromosome pair, number 15 as described previously by Schmid et al. (1982) (see Table 1, Fig. 1). This pair of chromosomes shows large secondary constrictions and satellites in most fish (Thorgaard 1976). Interphase cells showed either one or two nucleoli. The cells with one nucleolus could be the result of two NOR-bearing homologous chromosomes lying next to each other or only one of the two homologous chromosomes containing an active NOR. The former appears to be much more common than the latter, since all cells examined with 60 chromosomes had two NOR chromosomes.

Atlantic salmon

All of the Atlantic salmon examined had only one NOR-bearing chromosome pair, the small metacentric pair with satellites described previously by Hartley and Horne (1984) (see Table 1, Fig. 1). Interphase cells had either one or two nucleoli as in rainbow trout.

Brown trout

The Ag-NORs are located on the short arms of the two longest pairs of acrocentric or subtelocentric chromosomes (Table 1, Figs. 1 and 2*a*). They are readily identified since they are considerably longer than the other acrocentric chromosome pairs (Zenzes and Voiculescu 1975). There was variation in the number of chromosomes with active NORs, with most fish showing only two (see Table 2). No cells were observed with more than three active NORs. The number of

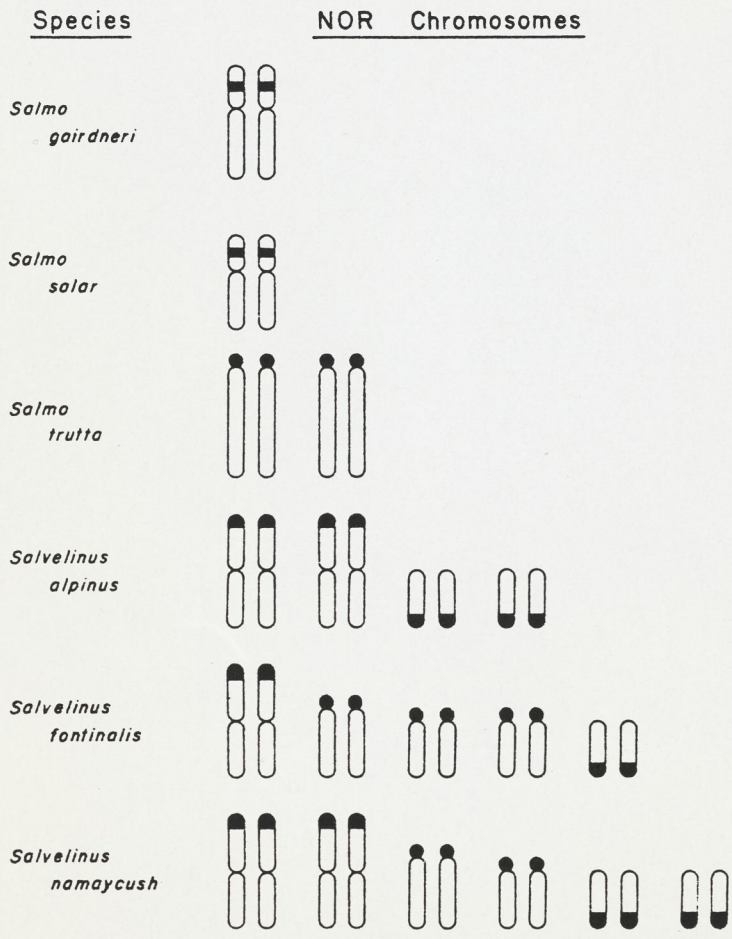

FIG. 1. Diagram of NOR chromosomes in different species of *Salmo* and *Salvelinus*.

nucleoli varied from one to two in some fish and from one to three in other fish.

Brook trout

The Ag-NORs are located at the telomeres of at least one metacentric chromosome pair, the telomeres of one acrocentric chromosome pair, and the short arms of several acrocentric chromosome pairs (Table 1, Figs. 1 and 2*b*). Because many of the acrocentrics cannot be distinguished from each other, only the minimum number of pairs involved can be estimated. The maximum number of Ag-NOR chromosomes observed in a single cell was 10, with 8 of these on short arms of acrocentrics, suggesting 4 chromosome pairs with NORs on short arms (Tables 2 and 3). The number of nucleoli per cell was variable from cell to cell and from fish to fish, with one to eight in different cells. Some fish varied from one to four, others from four to eight, and others were intermediate. The intraindividual variation appears to be mainly the result of several NOR chromosomes forming one nucleolus.

Lake trout

The Ag-NORs are located on at least six different chromosome pairs, the telomeres of two metacentrics and two acrocentrics and the short arms of at least two acrocentrics (Table 1, Figs. 1 and 2*c*). As in brook trout, different fish had different patterns of Ag-NOR staining. In lake trout the metacentric chromosomes can be distinguished by Q-banding, and sequential banding studies showed that at least two different metacentric chromosome pairs can have telomeric NORs. The majority of fish have only one metacentric chromosome with an Ag-NOR per cell. In the case of the acrocentric chromosomes, some cells were found with four NORs on the short arms and three on the telomeres, suggesting that a minimum of four different acrocentric chromosome pairs are involved. The two chromosome pairs with the Ag-NORs on the short arms include the largest acrocentric and a medium-sized acrocentric; therefore, these two can be distinguished. In the acrocentrics with telomeric NORs, associated heterochromatin was Q bright in some cases, so that in certain fish these

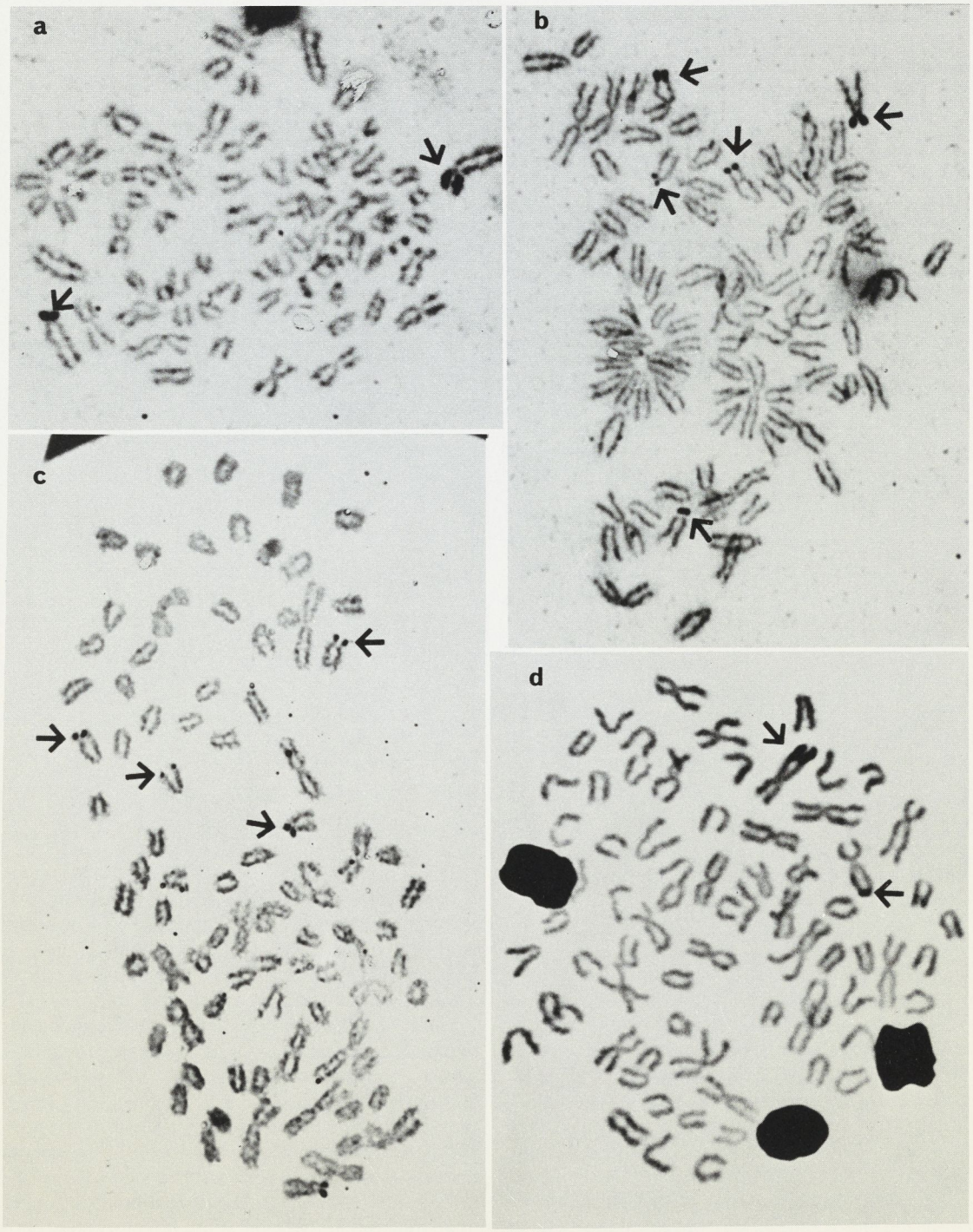

FIG. 2. Silver-stained metaphases of (*a*) brown trout, (*b*) brook trout, (*c*) lake trout, and (*d*) arctic char.

chromosome pairs could be distinguished. The number of active NORs per cell varied considerably, as in brook trout. The number of nucleoli per cell was also quite variable, ranging from one to eight in different cells in different fish, as in brook trout.

Arctic char

The Ag-NORs are located on the telomeres of at least two different metacentric chromosomes and at least two different acrocentric pairs, since some cells were found with three Ag-NORs on metacentrics and others with

TABLE 2. Variability in the maximum number of Ag-NORs per fish in different species

Species	Maximum no. of Ag-NORs/cell									Total no. of fish examined
	2	3	4	5	6	7	8	9	10	
Rainbow trout	10									10
Atlantic salmon	10									10
Brown trout	8	2								10
Brook trout			2	4	3	1				10
Lake trout			1	3	3	2	1			10
Arctic char		2	1		1					4

TABLE 3. Number of CM-A3* and Ag-NOR stained chromosome bands per cell in lake trout

Individual	Metacentric telomeric		Acrocentric telomeric		Acrocentric short arms		Total	
	CM-A3	Ag	CM-A3	Ag	CM-A3	Ag	CM-A3	Ag
1	1	1	5	2	3	2	9	5
2	—	—	2	2	4	2	6	4
3	1	1	3	1	5	3	9	5
4	—	—	2	2	4	2	6	4
5	—	—	2	1	5	3	7	4
6	2	2	4	2	2	2	8	6

*Chromomycin A3 counterstained with distamycin A.

three Ag-NORs on acrocentrics (Table 1, Figs. 1 and 2*d*). The number of nucleoli varied from one to six. The slides were made from adult tissue of only four individuals, in contrast with those from brook trout and lake trout in which preparations were made from 12 to 20 different fish. Therefore it is quite possible that the number of NOR chromosomes is comparable in all of the *Salvelinus* species. The metacentric chromosomes often had associated heterochromatin which was quinacrine bright.

Fluorochrome staining of NORs

In all species examined, the Ag-NOR regions showed negative staining with quinacrine. This is especially obvious in cases where the NORs are large. Figure 3 shows chromosomes from a lake trout metaphase which was sequentially stained with quinacrine and silver. The same chromosomal sites which are positive on silver staining are also stained by chromomycin A3 (CM-A3), as determined by sequential banding. In rainbow trout and Atlantic salmon most of the cells showed two chromosomes with CM-A3 positive bands, while in brown trout there was more variability. In the *Salvelinus* species generally more chromosomes per cell were positive on CM-A3 staining than Ag-NOR staining (see Fig. 4, Table 3), suggesting either that some of the rDNA is inactive in most cells, or that some additional sites are staining with CM-A3. If all of the CM-A3 sites represent rDNA, then the total number of chromosome pairs with NOR sites would be at least 10 in brook trout and at least 12 in lake trout. If this is the case, then polymorphism exists for the number of detectable rDNA sites as well as for NOR activity.

Discussion

All of the fish species studied for NORs previously have had only one NOR-bearing chromosome pair. Although most fish have a chromosome number of about 50, salmonids and some cyprinids have undergone a tetraploidization process, resulting in a DNA content and a chromosome arm number twice that of most other fish (reviewed in Gold 1979). The tetraploidization event is thought to have occurred more recently in salmonids than cyprinids since more enzyme loci have been silenced in cyprinids, and the cell size and amount of rRNA and rDNA per cell has been reduced in cyprinids (reviewed in Leipoldt and Schmidtke 1982; Whitt 1981). Among the salmonids, species of the genera *Oncorhynchus* and *Salmo* have undergone more chromosome fusions and a greater reduction in cell size compared with the *Salvelinus* species.

Recently Takai and Ojima (1982) have shown that the cyprinids, carp, and funas have only one NOR-bearing chromosome, and they propose that through unequal crossing-over between homeologous chromo-

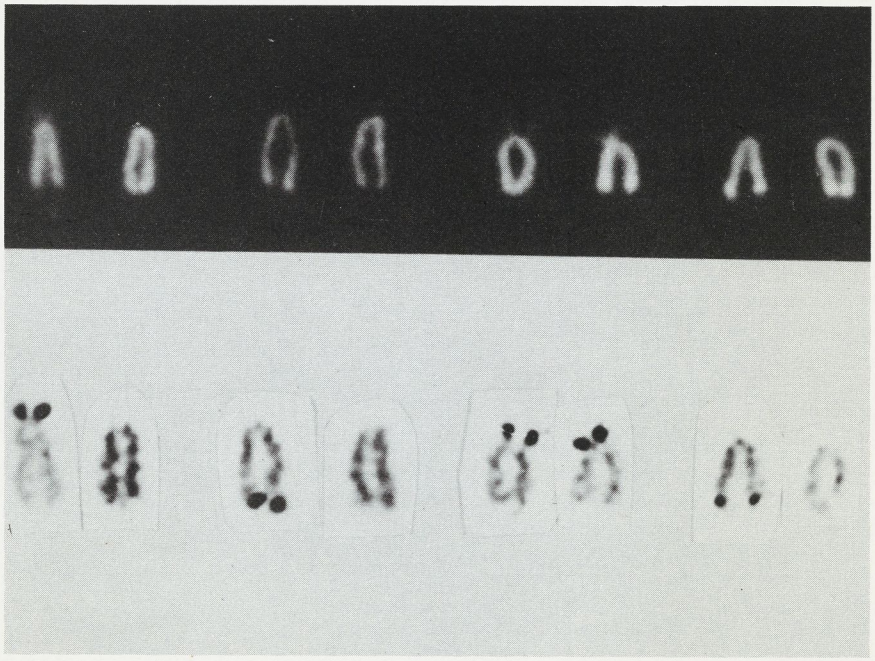

FIG. 3. NOR chromosomes from a lake trout metaphase sequentially stained with quinacrine and silver.

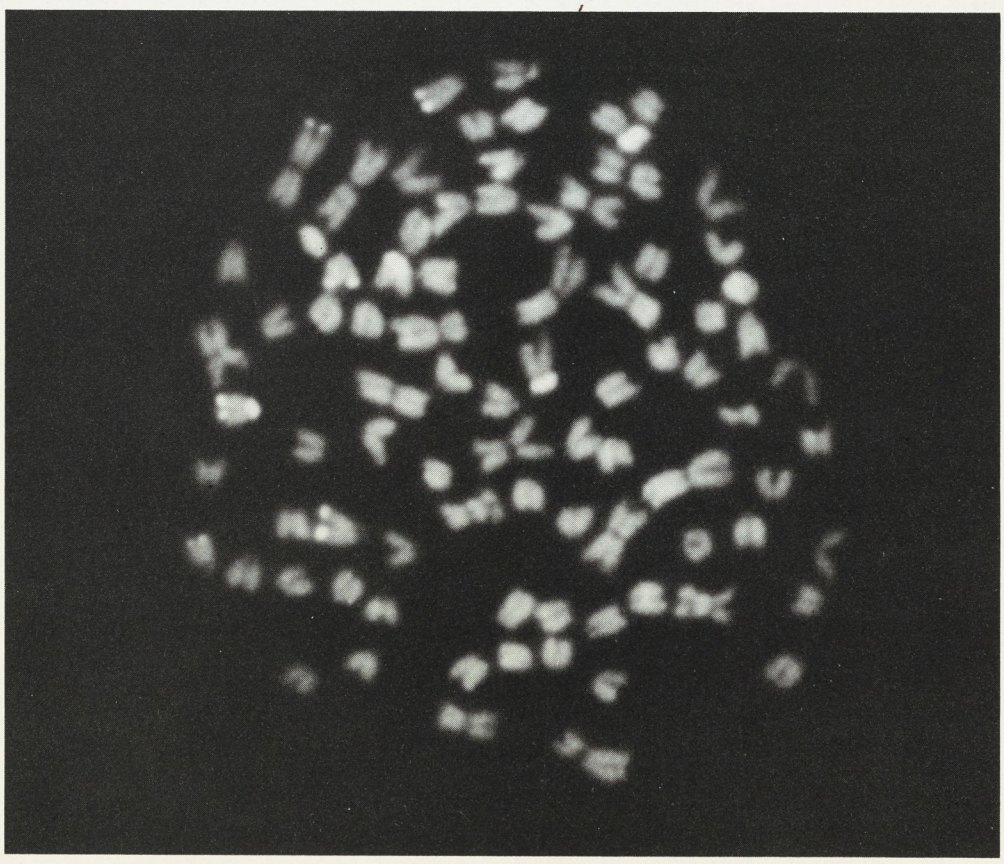

FIG. 4. Lake trout metaphase stained with chromomycin A3.

some pairs the number was reduced from two pairs to one after tetraploidization. In the case of rainbow trout ($2n = 60$) and Atlantic salmon ($2n = 56$), the reduction from two pairs to one could have occurred during a chromosomal fusion, since the NOR-bearing chromosome pair is a metacentric which was presumably derived from a fusion of two acrocentrics. In the case of brown trout ($2n = 80$) we find two NOR-bearing chromosomes, which are the two largest acrocentric chromosome pairs in the complement and could represent homeologous chromosomes. However, many fish have only two NOR chromosomes and the species could be in the process of a reduction of NOR chromosomes from two pairs to one. This could occur by unequal crossing-over during association with the nucleolus as proposed by Takai and Ojima (1982).

In the case of *Salvelinus* species there seems to be a trend toward chromosomal dispersion rather than fusion of the NORs. The number of chromosome pairs involved is much greater than the two which would have resulted after tetraploidization. It is interesting that these species have retained a relatively high chromosome number (82–84), larger cells, and a higher amount of rRNA per cell (Leipoldt and Schmidtke 1982). It seems possible that more rRNA is needed for larger cells and that one way of maintaining a high amount is by having a large number of chromosomal sites and multiple nucleoli. What determines whether the number of rDNA sites is multiplied or the amount of rDNA per site is increased is completely unknown, and evidence from primates suggests that amplification of rDNA sites can occur independently in different lineages (reviewed in Seuanez 1979). In the *Salvelinus* species more chromosome bands per cell were positive on CM-A3 staining than Ag-NOR staining. Some of these additional bands are probably inactive NOR regions, but others could be CM-A3 positive heterochromatin. In amphibians the rDNA stained brighter with CM-A3 than the associated heterochromatin, so that the two could be distinguished. *In situ* hybridization experiments are planned to determine whether all of the CM-A3 positive bands represent rDNA in the *Salvelinus* species.

In the *Salvelinus* species different fish vary considerably in the chromosomal location of the Ag-NORs and the size of the rDNA, as inferred from CM-A3 staining, as well as in the size and staining properties of the associated heterochromatin bands (Phillips and Ihssen 1983). Studies on the inheritance of these markers are in progress, and it is hoped that these parameters may be useful in stock identification of these fish.

Acknowledgements

The authors would like to thank Dr. James E. Wright, Jr., Pennsylvania State University, for the slides of adult arctic char and brook trout and for helpful suggestions concerning the research. This is publication No. 273 from the Center for Great Lakes Studies. This project was supported by a grant from the Great Lakes Fishery Commission.

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COLLEGE OF LETTERS AND SCIENCE
DEPARTMENT OF BIOLOGICAL SCIENCES

(414) 963-4214

January 24, 1987

Dr. Robert J. Behnke
Department of Fishery and Wildlife Biology
Colorado State University
Fort Collins, Colorado 80523

Dear Dr. Behnke:

Thank you so much for your prompt reply to my letter. I enclosed it along with the grant proposal which I just resubmitted. I also included a letter from Eldredge Bermingham, a post doc with Fred Utter, who has agreed to do restriction mapping of the rDNA from additional species in Salmo and Oncorhynchus for us with the probes we will send him. This should give us an additional data base with which to determine ancestral rDNA types. Bermingham is just completing a survey of mitochondrial DNA in salmonids which should be interesting. I might point out, however, that mitochondrial DNA has been found to be quite variable within lake trout, so we believe that the ribosomal DNA will be more useful for interspecies comparisons. Finally, Dr. Carroll Norden, of our department agreed to help out if we need him. I gave him some of your papers to read.

We obtained arctic char eggs from three stocks being maintained at the Rockwood Fish Hatchery north of Winnipeg this fall. As you previously indicated, the ones from Nauyuk Lake, Northwest Territories, should be the high arctic form (S. alpinus erythrinus). Do you know what part of the Northwest Territories these are from? In addition they sent some which they had obtained from Norway, which I assume would be S. alpinus alpinus, and some from Labrador, which I suppose is a zone of contact between the eastern and high arctic forms. The Labrador eggs were received last, and we don't have results from them. However, preliminary analysis of the other two stocks suggests that they are chromosomally different. The NORs are found at several additional sites in the Norweigian compared to the Northwest Territory stock. We should have these results analyzed sometime this spring.

Anne Kapuscinski, who is an assistant professor at University of Minnesota, and a recent graduate of the Fisheries department at Oregon state, is planning to go to Alaska next month for a workshop on pink and chum salmon. She is going to make arrangements for us to obtain pink salmon from several odd year stocks next year, and I have asked her to make inquiries regarding Dolly Varden and arctic char for me also. We definitely want to obtain the northern and southern Dolly Varden char from Alaska. Do you think it is important to sample more than one type of arctic char? If so, where should we obtain them? Have you made plans to go out there this spring?

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We are interested in obtaining the pink salmon from several west coast odd year stocks because we found a chromosome number polymorphism apparently involving both a fission translocation and inversion in the NOR chromosome pair in our Great Lakes stock which is derived from an odd year British Columbia stock. A simple fusion-fission polymorphism involving this same chromosome was also found by Russian scientists in pink salmon from Kamchatka. I am enclosing an abstract on this work in addition to a couple of reprints on papers on NORs in Salmoninae.

In response to your question regarding which Salmo species have the primitive NOR type (NORs on short arms of a medium sized acrocentric chromosome pair), it is probable that they all may have derived types. (This is in contrast to Salvelinus and Oncorhynchus in which more than one species appears to have the primitive type.) One could possibly consider that brown trout has the primitive type, since the NORs are on the short arms of an acrocentric pair in this species compared with interstitial locations on submetacentric pairs in the other species. However, the acrocentric pair with the NOR in brown trout is considerably larger than those with a similar NOR type in the other genera, and I believe it is probably derived from a tandem fusion betwen two acrocentrics. It will require G banding in order to determine homologies between these different chromosomes. Fortunately, chromosome changes in Salvelinus have been less extensive than in Salmo, and I have some new ideas regarding how to obtain G banding which I hope to try later this year.

I would be happy to collaborate with students in Alaska, if you find someone who is interested. Let me know if you have any specific advice regarding where we should sample arctic char or Dolly Varden in Alaska. I assume that Dolly Varden north of the Alaskan penisula would be the northern type. Thank you again for your letter and let me know if you go to Alaska this spring.

Sincerely yours,

Ruth B Phillips

Ruth B. Phillips

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DEPARTMENT OF BIOLOGICAL SCIENCES

(414) 229-4214

March 24, 1988

Dr. Robert Behnke
Department of Fishery and Wildlife Biology
Fort Collins, Colorado 80523

Dear Bob:

I will not be going to the Japanese symposium, although I did submit an abstract last year. I would like to go, but I can't afford the money or the time required during a semester when I have alot of obligations here.

I never did get the grant from NSF, which was rather discouraging. However after a period of several months without funding, I now have a 2 year grant from the Great Lakes Fishery Commission to study intraspecific variation in rDNA in lake trout. We are starting to compare the restriction maps of the rDNA from various salmonid species. I have extracted DNA from brook trout, lake trout and arctic char from several locations including Europe, but have not obtained any samples of the other Salvelinus species yet. If you are interested in collaborating with me, I would like to examine DNA from some specimens of the other Salvelinus species. Could you bring home some tissue from Japan? I believe DNA can be extracted from blood, if it has been kept cool for up to a week. Liver and other tissues must be frozen immediately and kept on dry ice until extraction. Do you have any contacts in Alaska who could obtain tissues from Dolly Varden?

I just submitted a manuscript to Copeia on the unpublished NOR work in Salvelinus, and am enclosing a copy of that manuscript, along with the abstracts from some other manuscripts which are also pending or in press. If you want to read any of the other manuscripts, let me know and I can send copies.

In order to prove the synapomorphies between chromosomes with NORs, we would have to do either silver staining of meiotic preparations from hybrids (which could be done) or G-banding. We hope to do either one or both of these in the future.

I wrote to Dr. Ueda a couple of months ago to ask him if he had ever done silver staining or CMA3 staining on S. leucomaenis, S. malma malma or S. malma miyabei. A few days ago I got some reprints from him, including one article in Japanese with an English summary:" The nucleolus organizer regions in the chromosomes of three species in the genus Salvelinus (Salmonidae)." He used the N banding technique, which is very similar to silver staining, and would stain active NORs. He found that the chromosome regions with satellites in S. leucomaenis and the S. malma species which I assumed were the NORs, were positive with N banding. He found multiple NORs in S. fontinalis and considerable intraspecific variability in that species which also agrees with my results.

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For your review, you might want to contact Lisa Ankenbrandt, a student at the School of Fisheries of the University of Washington in Seattle, who just completed her MS degree on mitochondrial DNA in several Salmo and Oncorhynchus species. Apparently she found that coho, chinook and rainbow trout were closely related. She found that the mtDNA was too variable to be useful in grouping the other Oncorhynchus species. This does not seem to be the case for rDNA, since we have found some highly conserved regions and other more diverged regions. I think the rDNA is going to be a very useful phylogenetic tool--too bad the NSF panel was so unsupportive! However, I am very interested in this project and have extracted DNA from several Salmo and Oncorhynchus species already.

Please let me know if you would be willing to assist me in obtaining samples for the rDNA work. I will be interested in seeing a copy of your abstract when you complete it, and hope that we will be at the same meeting sometime in the future.

Sincerely yours,

Ruth B Phillips

Ruth B. Phillips

P.S. Please note my address is now Department of Biological Sciences (the Zoology and Botany departments merged a couple of years ago).

Chromosome Polymorphisms in Great Lakes and Pacific Coast Stocks of Pink Salmon

Ruth B. Phillips, Department of Biological Sciences and Center for Great Lakes Studies, University of Wisconsin-Milwuakee, and
Anne R. D. Kapuscinski, Department of Fisheries and Wildlife, College of Forestry, University of Minnesota.

A polymorphism for chromosome number has been found in pink salmon from Lake Superior for two consecutive year classes (1984 and 1985). Although the majority of the fish have a diploid number of 52, as previously reported for this species, 15% of the population in both years had 2n=53, in which one of the metacentric chromosomes has undergone fission and then rearrangement to produce one acrocentric and one submetacentric chromosome containing the nucleolar organizer on the short arm (Phillips, Zajicek and Utter, 1986, and Phillips and Kapuscinski, in press). These fish are the progeny of a single planting in 1956 from an odd-year spawning the fall before of Skeena River, British Columbia fish (Kwain and Lawrie, 1981).

We have karyotyped only a small sample of odd year Pacific coast salmon from Washington State from a 1983 spawning, and all of these had the normal chromosome number of 52. However Gorshkov and Gorshkova (1981) reported that fish with 52, 53 and 54 chromosomes were found in a odd year Kamchatka population. This polymorphism resulted from a simple fission of one or two metacentric chromosomes, producing individuals with either 53 or 54 chromosomes. It seems likely that the chromosome constitution found in the Lake Superior fish represents a secondary rearrangement which occurred in a progenitor in which simple chromosome fission had resulted in 53 chromosomes, and we hope to karyotype individuals from the founder population in 1987. In 1986 we prepared karyotypes from a number of individuals from southern south east Alaskan stocks. All of these had the normal 2n=52 chromosome number, suggesting that the chromosome number polymorphism may be unique to odd year stocks.

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