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Stock Structure of Lake Trout (*Salvelinus namaycush*) from the Great Lakes Region as Determined by Chromosome and Isozyme Markers

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Abstract:

The genetic relationships between 8 different lake trout stocks were examined by analysis of data on the frequency of isozyme alleles and chromosome banding polymorphisms.Data was obtained for two consecutive years on 7 of the stocks for isozyme polymorphisms and 4 of the stocks for chromosome polymorphisms. Chromosome banding polymorphisms scored included quinacrine (Q) band size and intensity variants and nucleolar organizer region (NOR) polymorphisms as determined by chromomycin A3 (CMA3) staining. Fish were scored for 13 polymorphic isozyme loci. The electrophoretic and cytogenetic data differentiate these stocks similarly but not identically. The stocks can be placed into three groups on the basis of 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 somewhat different distribution of the CMA3 bands on specific chromosomes, which was consistent for two consecutive years. The stocks were very similar in the frequency of Q band variants, except for one polymorphism which was more common in the Michipicoten stock and another polymorphism which was rare in the Seneca Lake, New York stock. The largest electrophoretic genetic differentiation was between the Seneca Lake, New York population in the east and the stocks from the Great Lakes, with stocks from the same basin or geographic area being very similar. Stocks from the northern shore of the Great Lakes were differentiated from those originating

from more southerly areas in isozyme frequencies as well as in total number of CMA3 bands. A couple of consistent differences were found in the frequency of specific CMA3 bands for closely related stocks such as Gull Island Shoals and Marquette which were very similar electrophoretically. Fish from the Jenny Lake and Lewis Lake stocks which were transplanted from Lake Michigan to Wyoming 90 years ago had a high frequency of null alleles at two isozyme loci and a 50% reduction in the number of CMA3 bands. The frequency of null alleles and reduction in number of CMA3-NORs is most pronounced in the Jenny Lake stock, a hatchery stock with relatively poor hatching success, so that these genetic markers may reflect a reduction in biological fitness rather than simply a founder effect.

Introduction:

Early isozyme studies on lake trout indicated that relatively few loci were polymorphic, and only small differences in allele frequencies were found between fish collected from various locations in the Great Lakes region (Clayton and Ihssen, 1980, Todd, 1981). More extensive studies completed recently (Ihssen et al., 1986, and Leary et al., 1983) have shown that significant differences do occur between different populations, although these are not as large as those between different brook trout populations.

Identification of Q band chromosomal polymorphisms (Phillips and Zajicek, 1982), and CMA3 chromosomal polymorphisms (Phillips and Ihssen, 1984, 1985) in lake trout and the demonstration that these polymorphisms were heritable (Phillips and Ihssen,1984 and 1986,in press), suggested that they might be useful as genetic markers in population studies of lake trout.

In the present paper we report the results of chromosome studies on 8 different lake trout populations and compare the results from analysis of chromosome polymorphisms with those obtained with isozyme polymorphisms.

Materials and Methods:

Materials: Fish and eggs were obtained from lake trout from the following different hatchery stocks and wild populations. The Gull Island Shoals,(WI), Green Lake,(WI), Seneca Lake,(NY), Lewis Lake,(WY), Michipicoten Island,(ONT), Killala Lake,(ONT), and Manitou Lake,(ONT) trout were collected from wild populations, while the Marquette (MI) and Jenny Lake,(WY) trout were obtained from hatchery stocks.

Isozyme Methods: Samples were obtained from 4 different tissues from fingerlings or adult trout, and standard methods followed for electrophoresis of fish tissues.The 13 different loci which polymorphic were AAT-1,2,AAT-4, MDH-3,4, MuP, SOD, ME , GPI-1, GPI-2, LDH-4, PGM-1 and G3p-2.

Chromsome methods:Chromosome preparations were made from eggs.Eggs were incubated at 7 C and sacrificed at various stage of development,but best results were obtained from eggs about 160-180 degree days. Embryos were dissected from fertilized eggs and incubated in culture media with 25ug/ml colchicine as described previously (Thorgaard, 1981, Phillips and Zajicek, 1982) for 4 hours, exposed to hypotonic solution for 1 hour and fixed with Carnoy's fixative. Slides were prepared according to the methods of Kligerman and Bloom (1977).

For the Q banding, slides were stained in 0.5% Atebrin for 10 minutes and mounted in a saturated sucrose solution for fluorescence miscroscopy. To identify the CMA3-NORs, slides were stained in .5mg/ml chromomycin A3 for 2 hours,counterstained with distamycin A for 15 minutes and mounted in glycerin. To identify the silver-NORs, slides were stained using the method of Howell and Black (1980) modified by Gold (1983).

Slides were viewed with a Zeiss Universal microscope on bright field for the silver stained slides and with a BG 12 exciting filter and barrier filter of 47 or 50 for the fluorescent banded slides. Photographs were made using Kodak Technical Pan film with standard development using D-19 developer and 8 x 10 prints were made for karyotyping.

Scoring of Q bands: Two types of polymorphisms were scored: +/- variants (presence or absence of Q bands),and size variants which include differences in the size of the Q band on homologous chromosomes. The +/- variants were scored from karyotypes. Measurements of brightness of fluorescence followed the Paris conference (1971) .Intensely staining bands (level 4) were scored (+) and negatively staining bands (level 1) were scored (-). Size of Q bands was scored from negatives projected on a microfiche screen attached to a digitizer and computer. We have written a computer program specifically for analysis of chromosome banding patterns. With the aid of this program, measurement of chromosome arms relative to a standard in the cell (the short arm of the sex chromosome in lake trout),and classification of chromosomes into different structural groups and measurement of band size relative to a standard can be done quickly and accurately. The computer will combine data from several figures or from several fishes in order to print out a karyotype. The program is similar to that of Green et al, 1980,but has been expanded considerably to perform chromosome banding analysis.

Scoring of CMA3 bands: Size of CMA3 bands was scored in the same way as size of Q bands. For each cell, the CMA3 bands and the chromosomes on which they were found were measured, so that data was obtained for each cell on the total # of CMA3 bands/ cell, the total CMA3 band length in each cell, the relative size of each chromosome with CMA3 bands, the position of the CMA3 band on the chromosome and the size of the CMA3 band on each chromosome.

Results:

Description of chromosome markers found:

Q Band Variants: Q bands are found on up to 5 pairs of metacentric chromosomes and 2-3 pairs of acrocentric chromosomes. The metacentric chromosomes can be distinquished on the basis of size and banding pattern (see figure 1), but the acrocentric chromosome pairs with Q bands can not be reliably distinguished from each other. Variation in the size of the Q bands on homologous chromosomes was found for all of the Q bands, but significant differences were found primarily in the larger Q bands, which were on chromosomes 2, 7 and 8. The differences in band size have been shown to be consistent for an individual fish and heritable in crosses (Phillips and Ihssen, 1986, in press).

Variations in the presence or absence of Q bands on homologous chromosomes (+/- variants) were found primarily for bands on chromosomes 2, 5 and 8. In the case of chromosome 2, there is a difference between male and female lake trout in the presence or absence of a bright Q band on this chromosome, (Phillips and Ihssen, 1985) such that females have a bright band on both chromosomes, and males have one chromosome (the Y) which lacks this band. This chromosome pair can always be identified because it is the only large submetacentric chromosome in the karyotype. The majority of fish have two bright telomeric bands on chromosome 8, and lack bands on chromosome 5, but individuals lacking heterozygous for these +/- variants have been found and these markers have been shown to be heritable in crosses (Phillips and Ihssen, 1986, in press).

CMA3 Variants:

These bands are found at three different types of locations: telomeric bands on metacentric chromosomes, telomeric bands on acrocentric chromosomes,and bands comprising the entire short arms of acrocentric chromosomes. These bands are found on at least 10 different chromosome pairs in lake trout. Many individuals have a CMA3 band on only one of the two homologous chromosomes in a given chromosome pair. Variations occurred in the total number of CMA3 bands, their chromosomal location and in the size of the bands.

For the metacentric chromosomes, most fish have a band on only one homologue of one of the following chromosome pairs: 1,2,3, or 4 .A few individuals have been found heterozygous for a band on chromosomes 7 and 8. The acrocentric chromosomes cannot be individually distinguished, although they fall into 3 size classes. Most of the CMA3 bands are on chromosomes in the largest sized class or the medium sized class. Therefore we divided the bands on the acrocentric chromosomes into the following groups: bands on the short arms of a large sized acrocentric, bands on the short arms of a medium sized acrocentric, bands on the telomeres of a large sized acrocentric and bands on the telomeres of a medium sized acrocentric. A fifth class included acrocentric chromosomes with bands on both telomeres and short arms (doubles).

The number of CMA3 bands and their chromosomal location has been found to be consistent for a given individual and certain marker chromosomes which can be unambigously indentified have been shown to be heritable in crosses in which haploid and diploid progeny were scored (Phillips and Ihssen,1984,see Table 1).

Frequency of Q Band Chromosome Variants

The different stocks were all quite similar with respect to Q band polymorphisms (see Table 2). For +/- variants , the Seneca Lake stock had a lower frequency of Q bands on chromosome 5 compared with the others and the Michipicoten stock had a lower frequency of Q bands on one arm of chromosome 8 than the other stocks. This shows up in the difference in total Q bands which is lower for this stock. Although size variants were quite common, there were no statistically significant differences in total band length for different chromosomes as measured by computer. Visual scoring of band size from karyotypes originally suggested that that some stocks might have a higher frequency of chromosomes with large bands, but results of computer scoring of relative band size did not reveal significant differences between stocks.

Frequency of CMA3 Band Chromosome Variants

Differences were found between the different stocks in the total number of CMA3 bands, the total band lengths, and in the chromosomal location of the bands (see Table 3). The largest difference between stocks in number of CMA3 bands and total amount of banded material was between the Wyoming stocks originally from Lake Michigan which had an average number of bands equal to 4.4, compared with 7.4 to 10.4 for the other stocks. Secondarily there was a difference between stocks from the northern shore of the upper lakes (average total bands =9.8) and stocks from the southern shore(average total bands =7.5).

Each stock had a unique combination of CMA3 variants. These were reproducible when scored for two consecutive years (see Table 4). For example,the Gull Island Shoals and Marquette stocks which have a very similar average number of CMA3 bands per individual and are both from the southern shore of Lake Superior at different locations were significantly different in the frequency of two CMA3 bands. The band on the long arm of chromosome 2 was present on one homologue in 22% of the Marquette fish ,but completely absent from the Gull Island Shoals fish. The double banded acrocentric chromosome was found in 41% of the Gull Island Shoals fish, but only 4% of the Marquette fish. The Wyoming stocks had a very low frequency of bands on both chromosomes 1 and 2. The Michipicoten stock had a much higher frequency of CMA3 bands adjacent to Q bands than the other stocks.

Comparison of Chromosome Data and Isozyme Data

Electrophoretic data on the same stocks is given in Table 5. The electrophoretic and cytogenetic data differentiate the stocks similarly but not identically. The largest electrophoretic difference is between the Seneca Lake New York stock and the other stocks. One of the two differences in Q bands was found between the Seneca Lake stock and the others and CMA3 differences were also found, but they were not greater than those between the other stocks. Secondarily the electrophoretic data distinguished the stocks from the northern shores of the upper lakes from those from more southerly locations and this also was reflected in the differences found in the total number of CMA3 bands. The northern stocks had the highest number (average of 9.8), the southern Lake Superior stocks had a medium number (average 7.5)

and the Wyoming stocks originally from Lake Michigan had the lowest number (average 4.5) . These Wyoming stocks are very similar to the southern Lake Superior stocks with the exception that they had a high frequency of null alleles at two loci. The presence of null alleles and 50% reduction in CMA3 bands both suggest a decrease in genetic diversity in these stocks.

Discussion:

Data in this paper show that the frequencies of chromosome banding polymorphisms in lake trout are stock specific, just as the frequencies of isozyme alleles have been found to be characteristic of a given stock in many fish species. In fish species such as rainbow trout, which have a variable diploid chromosome number, consistent differences in the diploid number of chromosomes have been found between geographically separate populations (Thorgaard, 1983),but no population studies on chromosome banding polymorphisms in fish have been reported.

Differences in the total number and location of chromosome bands revealed by quinacrine staining (Q bands) and chromomycin A3 staining (CMA3 bands) were found between different stocks. Few differences were found between stocks in Q band polymorphisms, while each stock had a unique combination of CMA3 variants. Thus it seems that the number and location of the Q bands is primarily a species characteristic, while considerable intraspecific variation occurs in CMA3 bands.

The Q bands represent constitutive heterochromatin, which almost always varies in amount and chromosomal distribution between related species and often shows extensive variation in size of bands between homologous chromosomes (reviewed in John, 1981).In humans, racial differences have been found in the frequency of variable Q bands on several chromosomes and in the sizes of bands on particular chromosomes (reviewed in Verma and Dosik, 1980).The variations in the presence or absence of bands probably reflect differences in the sequence of the satellite DNAs, while differences in size may be produced by unequal crossing over or amplification events. A recent quantitative study of C bands in two human races,(Cavalli et al, 1985),showed that although the mean size of the C band on chromosome 9 was larger in Caucasians and the mean size of the C band on the Y chromosome was larger in Japanese, the average total C band length was the same in both races. These results and those of our study in lake trout suggest that the amount of heterochromatin is under strict control in these species and may have a biologically important function.

In contrast to the Q bands which were found in the same location on homologous chromosomes in most individuals, considerable variation in the location of the CMA3 bands was found, with many fish have detectable bands on only one of the two homologues. CMA3 stains the nucleolar organizer regions which contain the ribosomal RNA genes in many amphibian (Schmid, 1982) and fish species including salmonids ,(Phillips and Ihssen, 1983,

Phillips and Ihssen, 1985), cyprinids,(Amemimya,1985) and perch (Mayr et al, 1985). Results from amphibians and our results from salmonid fishes are consistent with CMA3 staining the nucleolar organzer regions(NORs) which contain the ribosomal RNA genes regardless of activity, while silver staining identifies the active NORs. Salmonid fishes are believed to be ancestral tetraploids and they have a much larger number of the ribosomal RNA genes compared with related diploids. We believe that in lake trout some of these genes are being lost from the genome and this accounts for differences in the total number of bands and differences in their location. If this is the case, it is not surprising that the CMA3 markers are able to differentiate some stocks which are electrophoretically very similar.

The results reported here probably underestimate the chromosomal differences since it was not possible to distinguish all of the acrocentric chromosomes from each other,and data on up to 6 different chromosome pairs had to be lumped into two groups. Because not all of the chromosomes could be identified, it is difficult to make accurate estimates of genetic similarities between groups, because some groups which appear similar might have had differences in the bands on these chromosomes. We are exploring the possibility of using replication banding to distinguish these chromosomes.

Analysis of both chromosomal and electrophoretic markers in these stocks indicate that these lake trout stocks are genetically different from each other. Since most of the

differences are in frequency of particular markers, rather than presence or absence of markers, these stocks have probably differentiated from each other since the last ice age.

The Wyoming stocks showed evidence of reduction in genetic diversity from both the electrophoretic and the chromosome data. Two null alleles were found in these stocks, which could have a negative effect on fitness. In addition a greater than 50% reduction in CMA3 bands which is probably equivalent to a 50% reduction in ribosomal RNA genes was found in these stocks. Although these fish probably have more copies of these genes than they need, a reduction of this magnitude could have physiological effects, possibly on stockpiling of ribosomes in the oocyte. These reductions in genetic diversity were most pronounced in the Jenny Lake stock, which has a lower hatching success than the other stocks (Binkowski, personal communication).

Table 1. Inheritance of CMA3 Polymorphisms in Lake Trout in Gynogenetic Haploid Progeny

CMA3 Marker	Female Genotype	Haploid Progeny +	-	N
Chrom # 4-Lg T	+/+	20	-	20
Chrom # 9-Db	+/-	11	9	20
Chrom # 9 Xlg T	+/-	9	6	15

Table 2. Frequency of Q Band Polymorphisms in Lake Trout Stocks

+/- Variants	Lk Michigan	Finger Lks	Upper Lks (So)		Upper Lks (No)		
Proportion present	Lewis Lk.	Seneca Lk	G.I. S.	Mar.	Mich.	Man.	Kill
Chrom 5	0.30	0.05	0.29	0.40	0.43	0.40	0.41
Chrom 7-2	0.92	0.95	0.89	0.93	1.00	1.00	1.00
Chrom 8-2	0.97	0.92	0.79	0.84	0.58	0.69	0.73
Av Total # Q bands on metas	13.6 (1.0)	13.7 (0.9)	13.1 (1.1)	13.6 (1.1)	11.3 (1.8)	13.6 (1.0)	13.5 (0.8)
Av Total # Q bands	16.0 (2.2)	16.8 (1.7)	16.4 (1.4)	16.2 (1.2)	15.6 (2.2)	16.4 (1.8)	16.2 (1.9)
Av Total relative band length	5.5 (1.2)	4.9 (1.4)	4.6 (0.9)		5.2 (1.0)		
Size Variants							
Chrom 2-bd 1	.52 (.1)	.45 (.1)	.40 (.1)		.52 (.1)		
Chrom 7-bd 1	.26 (.1)	.24 (.06)	.22 (.04)		.27 (.05)		
Chrom 7-bd 2	.47 (.1)	.38 (.1)	.42 (.1)		.48 (.1)		

Numbers in parenthesis are standard deviations. Band lengths were measured only for four stocks.

Table 3. CMA3 Polymorphisms in Lake Trout Stocks

	Lake Michigan		New York	Lake Superior-South			Lake Sup. Hur.(North)		
	J.L.	LL.	S.L.	G.I.S.	Mar.	Gr.L.	Mich	Man	Kill
Av. # bands	3.9 ±1.4	4.9 ±2.3	8.1 ±1.2	7.7 ±2.0	7.4 ±2.2	6.5 ±1.2	10.4 ±2.1	9.7 ±2.5	10.2 ±1.6
Av. Tot. band leng.	1.5 ±.6	2.1 ±.9	3.1 ±1.1	3.0 ±.8	2.5 ±.9	2.6 ±.6	4.6 ±1.2	3.2 ±.9	
Av bands on metas	.17 (.04)	.78 (.16)	1.2 (.15)	.92 (.12)	1.1 (.15)	.41 (.06)	1.13 (.11)	.62 (.06)	
Av bands on acro-sa	1.55 (.40)	2.30 (.46)	4.06 (.50)	3.48 (.45)	3.44 (.46)	3.25 (.50)	5.21 (.51)	5.0 (.53)	
Av bands on acro-t	1.95 (.50)	1.5 (.31)	2.6 (.32)	3.05 (.38)	2.80 (.38)	2.50 (.38)	3.7 (.36)	3.6 (.37)	
av bands on db acr	.28 (.07)	.14 (.03)	.27 (.03)	.35 (.05)	.04 (.005)	.33 (.05)	.36 (.03)	.38 (.04)	
av bands on specific metas:									
#1	.06	.00	.23	.32	.33	.08	.21	.08	
#2	.00	.07	.17	.00	.22	.00	.21	.15	
#3-6	.11	.71	.67	.54	.56	.33	.71	.31	
#7-8	.00	.00	.10	.05	.00	.00	.00	.08	
% adj Q on chrom 4	0%	10%	17%	20%	16%	25%	60%	50%	
Sample Size	18	14	32	37	45	12	14	14	7

*Numbers in parthenesis refer to proportion of bands at a particular location

Table 4. CMA3 Polymorphisms in Different Year Classes of Lake Trout Stocks

	G.I.S.		Marquette		Seneca Lake, NY	
	83	84	84	85	83	84
Av # bands	8.8	7.1	7.3	7.6	8.5	7.6
Av Tot. band length	3.53	2.67	2.82	2.15	3.16	3.04
Av bands on metas	1.0 (.11)	.87 (.12)	1.31 (.18)	1.11 (.15)	1.41 (.17)	.93 (.12)
Av bands on acro-sa	3.29 (.37)	3.61 (.51)	3.43 (.47)	3.44 (.46)	4.18 (.49)	3.93 (.51)
Av bands on acro-t	3.92 (.45)	2.34 (.33)	2.50 (.34)	2.80 (.38)	2.65 (.31)	2.54 (.33)
Av bands on acro-db	.64 (.07)	.17 (.02)	.04 (.005)	.04 (.005)	.29 (.03)	.23 (.03)
Av bands on specific metas						
#1	.43	.26	.35	.32	.35	.15
#2	.00	.00	.23	.21	.23	.08
#3-6	.50	.57	.73	.32	.71	.62
Sample Size	14	23	26	19	17	13

*Numbers in parenthesis refer to proportion of total bands at a particular location

Table 4. CMA3 Polymorphisms in Different Year Classes of Lake Trout Stocks

	G.I.S.		Marquette		Seneca Lake, NY	
	83	84	84	85	83	84
Av # bands	8.8	7.1	7.3	7.6	8.5	7.6
Av Tot. band length	3.53	2.67	2.82	2.15	3.16	3.04
Av bands on acro-sa	3.29 (.37)	3.61 (.52)	3.43 (.47)	3.47 (.46)	4.18 (.49)	3.93 (.52)
Av bands on acro-t	3.92 (.45)	2.43 (.34)	2.50 (.34)	3.21 (.42)	2.65 (.31)	2.54 (.33)
Av bands on acro-db	.64 (.07)	.17 (.02)	.04 (.005)	.05 (.007)	.29 (.03)	.23 (.03)
Av bands on metas	1.0 (.11)	.87 (.12)	1.31 (.18)	.85 (.11)	1.41 (.17)	.93 (.12)
Av bands on specific metas						
#1	.43	.26	.35	.32	.35	.15
#2	.00	.00	.23	.21	.23	.08
#3-6	.50	.57	.73	.32	.71	.62
#7-8	.07	.04	.00	.00	.12	.08
Sample Size	14	23	26	19	17	13

*Numbers in parenthesis refer to proportion of total bands at a particular location

Table 5.

FREQUENCY OF THE 100 ALLELE (100 AND 85 ALLELLE FOR AAT-1,2) AT 12 POLYMORPHIC LAKE TROUT LOCI.

	POPULATIONS							
LOCUS	GULL (40)[1]	JENNY (80)	LEWIS (74)	KILLALA (161)	MARQUETTE (89)	SENECA (95)	MICHIPICOTEN (60)	MANITOU (202)
AAT-1,2	0.519	0.484	0.507	0.478	0.444	0.715	0.550	0.593
AAT-1,2[2]	0.456	0.472	0.473	0.508	0.494	0.285	0.450	0.406
AAT-4	1.000	0.888	0.799	1.000	1.000	1.000	1.000	1.000
MDH-3,4	1.000	1.000	0.997	0.842	0.969	0.984	0.900	1.000
MUP	0.975	1.000	0.959	0.907	0.938	0.984	0.900	1.000
SOD	0.937	0.950	0.959	0.972	0.983	1.000	1.000	0.993
ME	0.975	1.000	1.000	1.000	1.000	0.853	0.983	0.978
GPI-1	0.988	1.000	1.000	1.000	0.994	1.000	0.950	0.906
GPI-2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.953
LDH 4	1.000	1.000	1.000	1.000	1.000	1.000	0.867	1.000
PGM-1	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000
G3P-2	1.000	1.000	1.000	1.000	1.000	0.932	1.000	1.000

[1]SAMPLE SIZE.

[2]AAT-1,2 (85) ALLELE.

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