GREAT LAKES FISHERY COMMISSION

1983 Project Completion Report[1]

Application of Chromosome Banding Techniques to Stock
Identification in Lake Trout

by:

Ruth B. Phillips

Dept. of Biological Sciences and
Center for Great Lakes Studies
University of Wisconsin-Milwaukee
Milwaukee, Wisconsin

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Completion Report on Year 1 of Contract Initiated Sept 1983:

Application of Chromosome Banding Techniques to Stock Identification
in Lake Trout

Principal Investigator: Ruth B. Phillips
                        Dept. of Biological Sciences and
                        Center for Great Lakes Studies
                        University of Wisconsin-Milwaukee
                        Milwaukee, Wisconsin

Collaborator: Peter E. Ihssen
              Fisheries Research Branch
              Ontario Ministry of Natural Resources
              Maple, Ontario

Summary:

     Chromosome preparations were obtained from 25 individuals from 7
different lake trout stocks and Q banding and CMA3 (NOR) banding
techniques applied. Visual scoring of Q band variants and CMA3
variants from photographs was done on 16-25 individuals from the
different stocks. Preliminary analysis of this data shows differences
in frequency of banding variants between the different stocks. Each
stock appears to have a unique pattern of banding variants when
information from both banding techniques is used. Comparison with
results from isozyme electrophoresis on several of the same stocks
shows that the frequency of +/- Q banding variants is in close
agreement with results from electrophoresis, in showing differences
primarily between fish populations which probably used different
glacial refugia during the last ice age. The other chromosome
techniques showed differences between populations which are from the
same general geographic region. During the second year we plan to
sample some of the same stocks a second time in fall, 1984 in order to
determine the stability of the chromosome marker frequencies from year
to year. We are also in the process of setting up a microcomputer
based karyotype analysis system for the faster and more accurate
scoring and analysis of data. We plan to develop a discriminant
function to differentiate the different stocks using chromosome
variants if this is appropriate.

Introduction:

In the original grant request we proposed three major objectives for the two year period. The first objective was to apply the Q band and NOR staining techniques to chromosome preparations from a sample of approximately 25 individuals from several of the major stocks of lake trout. This has been completed for 7 lake trout stocks: Seneca Lake, Lewis Lake, Michipicoten Island, Gull Island Shoals, Killala Lake, Manitou Lake and Marquette. with sample sizes from 16 to 25.

The second objective was to determine the frequency of these chromosome markers in 50 individuals from 2 hatchery and 2 wild stocks. This will be accomplished the second year by scoring an additional 25-30 individuals from 4 of the stocks studied in 1983: Seneca Lake, Gull Island Shoals, Lewis Lake and Marquette.

The third objective was to analyze the data and compare it with data from isozyme electrophoresis. This was been done for the data obtained in 1983.

Results:

Q Band Chromosome Markers:

In order to use new genetic markers in population studies, one must first show that they are stable and inherited in the expected Mendelian manner. We have completed a series of experiments which showed that the Q band markers are inherited as expected and have submitted a manuscript on this work for publication in Genetics:Inheritance of Q Band Chromosomal Polymorphisms in Lake Trout" . During the analysis of the crosses, we identified the sex chromosomes in lake trout. A poster paper on this research was presented at the joint meeting of the American and Canadian Genetics Societies in Vancouver in August, 1984 and a manuscript on this work has been accepted for publication in Cytogenetics and Cell Genetics:"Identification of Sex Chromosomes in Lake Trout:(Salvelinus namaycush").

We have found two types of Q band polymorphisms in lake trout,those which represent qualitative differences in the bands(bright vs dull or negative staining with quinacrine) and those involving quantitative differences in the size of the bands. The qualitative markers are thought to represent differences in the DNA sequence of the repetitive DNA making up the bands, while the quantitative differences represent differences in the total amount of repetitive DNA at that site.

Visual scoring of these two types of Q band markers in the various lake trout stocks collected in the fall of 1983 is complete and the results showed difdferences in frequency of these markers in the different stocks.

Q Band +/- Markers:

The main difference was between the Seneca Lake stock and the other stocks which are all originally from the upper Great Lakes region. The frequency of bright Q bands on chromosomes 5,6 and 8 is significantly lower in the Seneca Lake stocks than the other stocks. These results correspond to those obtained from isozyme electrophoresis which showed significant differences in isozyme frequencies between the Seneca Lake stock and the stocks found in the upper Great Lakes.

Q Band Size Markers:

Visual scoring of size markers showed significant differences in size of Q bands in different stocks. Most of the variation in Q band size occurs on the bands on chromosomes 2, 7 and 8. A significantly higher frequency of large bands on these chromosomes was found in the Lewis Lake, Manitou Lake and Killala Lake stocks compared with the Seneca Lake, and Lake Superior stocks: Marquette and Gull Island Shoals. The significance of these findings should be clear after fish from the same stocks are analyzed for a second year and with the more accurate computer based methods.

For the quantitative markers the computer scoring of bands would be more accurate than visual scoring of small medium and large sizes, but this is very time consuming and not very accurate when done from photographs. Therefore we have been spending considerable time setting up a microcomputer based image analysis system using a sonic digitizer to make measurements from negatives projected on a microfische screen. We obtained a computer program for making chromosome measurements from another laboratory, but unfortunately have had to completely rewrite it for our purposes. The measurement part of the program has just been completed and is operational, but other parts which will make comparisons between different chromosome sets and instruct a printer to draw diagrams are still being rewritten. When this is complete, we plan to rescore the data from 1983 and score the data from fall, 1984.

NOR (Nucleolar Organizer Region) Markers:

The NORs are usually distinguished with a silver staining method which stains only the NORs active at the previous cell division. For genetic analysis it would be desirable to have a technique which reveals all of the NORs regardless of their activity. In amphibians, it has been found that the fluorescent dye, chromomycin A3 (CMA3) stains the inactive and active NOR regions. In a preliminary study on NORs in salmonid fish, we have obtained evidence that this is also the case for these fish. A manuscript describing this work has been submitted to Chromosoma. CMA3 bright bands are found at three types of chromosomal sites in lake trout: short arms of acrocentrics, telomeres of acrocentrics and telomeres of meetacentrics. The total number of sites and the location of these sites as well as the size of the bands varies from individual to individual. For a given individual the pattern is constant. in order to use these CMA3 bands as genetic markers it is necessary to show that the differences in the bands are heritable. We obtained some data showing inheritance of the CMA3 markers last fall, but more crosses are needed to study all of the markers. Last fall we discovered that haploids are ideal material for studying inheritance and frequency of these markers, since they have half of the normal chromosome number and only those chromosomes from the maternal parent. Haploids can be routinely produced from green eggs and sperm and we plan to use them extensively in the fall of 1984.

The computer based system would be a great advantage for scoring CMA3 markers because the relative sizes of the chromosomes with the bands as well as the sizes of the bands themselves could be compared from different fish. We plan to rescore the data from 1983 along with the new slides from 1984 using this technique.

Preliminary visual scoring of qualitative and quantitative CMA3 markers showed that each stock had a unique pattern of CMA3 bands. The total number of bands was highest in the Lake Superior stocks (Michipicoten Island, Gull Island Shoals and Marquette) and lowest in the Lewis Lake stock,originally from Lake Michigan,and in the Seneca Lake stock. A number of cases of variants which were present at a relatively high frequency in one stock, but at a low frequency or absent in other stocks was found. Since there is considerable variability between individuals within a stock for these markers, it will be especially important to score these for a second year in the same stocks.

Since the CMA3 markers represent differences in the rDNA, these markers might be more efficiently scored using a DNA method. In a preliminary study in collaboration with my colleague Peter Wejksnora in the UWM Biological Sciences Department,the rDNA of lake trout was isolated and found to occur in two molecular forms, a major 22kb and a minor 19-21kb form.(Popodi, masters thesis, 1984 and Popodi et al, 1984). Twenty individual fish from different lake trout stocks were examined and variations in the size of the minor form were found. The advantage of DNA markers is that can be determined on frozen tissue and can measure small differences in the genetic material.The rDNA occurs in multiple copies, so sufficient DNA is present in small fish to determine their genotype. We are interested in applying this technique to the problem of lake trout stock identification in the future.

Comparison With Data From Isozyme Electrophoresis

A survey of lake trout stocks using the methods of isozyme electrophoresis is in progress in the laboratory of Peter Ihssen and a report on isozyme comparisons of the Jenny Lake and Lewis Lake trout stocks by Fred Allendorf was made available to me by the Colorado office of the US Fish and Wildlife Service earlier this year. Results from the laboratory of Peter Ihssen show that lake trout stocks fall into four major groups: a western group (Clearwater, Manitoba), an eastern group (Seneca Lake,NY and Lake Simcoe, Ontario),an upper Great Lakes group (Manitou Lake, Marquette, Gull Island Shoals, Michipicoten Island, Killala Lake etc)and a group in the Haliburton Islands region (McDonald Lake etc) where a glacial river existed during the last ice age. Thus the major groups seem to correspond to glacial refugia of the last ice age. Results from Allendorf´s laboratory on the Wyoming stocks, Jenny Lake and Lewis Lake, derived from Lake Michigan showed that these fish are very similar to those from the Upper Great Lakes region except for the occurrence of null alleles at two loci in the Jenny Lake stock,suggessting possible loss of genetic variability due to inbreeding in this stock.

The results from the Q band +/- variants parallel those from isozyme electrophoresis, in showing a difference between eastern(Lake Seneca) and Upper Great Lakes stocks. Using the Q band size variants, differences were seen between several of the stocks which are similar in isozyme frequences: the Lewis Lake, Manitou Lake and Killala lake stocks had signficantly higher frequencies of large Q bands than the Lake Superior Stocks(Gull Island Shoals, Marquette and Michipicoten Island),and the Lake Seneca stock had the lowest frequency of large Q bands. With the CMA3 variants, every stock had a unique pattern of variants. The Lake Superior stocks were similar in total number of CMA3 bands. Results from fall of 1984 should yield important information on how stable the frequency of these markers is from year to year.

1984 Papers, Abstracts, Manuscripts:

Phillips, Ruth B and Ihssen, Peter E, Identification of sex chromosomes in lake trout (Salvelinus namaycush).Cytogenetics and Cell Genetics (in press)

Phillips, Ruth B, Zajicek, Kerry D., and Utter, Fred M., Q band chromosomal polymorphisms in chinook slamon (Oncorhynchus tshawytscha). Copeia (in press)

Phillips, Ruth B., and Ihssen, Peter E. Chromosome banding in salmonid fish: nucleolar organizers in Salmo and Salvelinus (submitted to Canadian Journal of Genetics and Cytology)

Phillips, Ruth B., and Ihssen, Peter E. Inheritance of Q band chromosomal polymorphisms in lake trout (submitted to Journal of Heredity).

Popodi, E.M. Kattar, P., Phillips, R.B., and Wejksnora, P.J. Ribosomal RNA genes of lake trout.(American Society for Biological Chemistry meeting abstract).

Phillips, R.B and Ihssen, P.E., Inheritance and frequency of chromosome banding polymorphisms in lake trout(Genetics Societies meeting abstract).

Phillips, R.B., and Ihssen, P. E., Chromosome banding difference between the X and Y chromosome of lake trout(Genetics Societies meeting abstract)

Zajicek, K.D. and Phillips, R.B. Mitotic inhibition and anaphase aberrations in rainbow trout embryos treated with MMNG and gamma radiation(Genetics Societies meeting abstract).

Proposed Work for October ,1984-December 1985.

Major Objective--remains the same, to apply chromosome banding techniques to the problem of stock identification in lake trout of the Great Lakes region.

Specific Objectives:

Objective 1-To score an additional 20-30 individuals from 4 of the stocks studied last year (Seneca Lake, Lewis Lake, Marquette, Gull Island Shoals,if possible) to obtain a better estimate of fequency of chromosome markers and to determine their stability from year to year. To add the Green Lake stock to the stocks studied.

Objective 2- To develop and use improved methods for scoring and analysis of chromosome markers using a microcomputer based image analysis system.

Objective 3-To obtain more inheritance data on the CMA3 markers using haploids for analysis.