

THE UNIVERSITY OF MANITOBA

GENETIC STUDIES OF PROTEIN VARIANTS AND THEIR USE IN A
ZOOGEOGRAPHIC STUDY OF LAKE WHITEFISH, COREGONUS CLUPEAFORMIS (MITCHILL)
IN WESTERN CANADA

by

WILLIAM GILBERT FRANZIN

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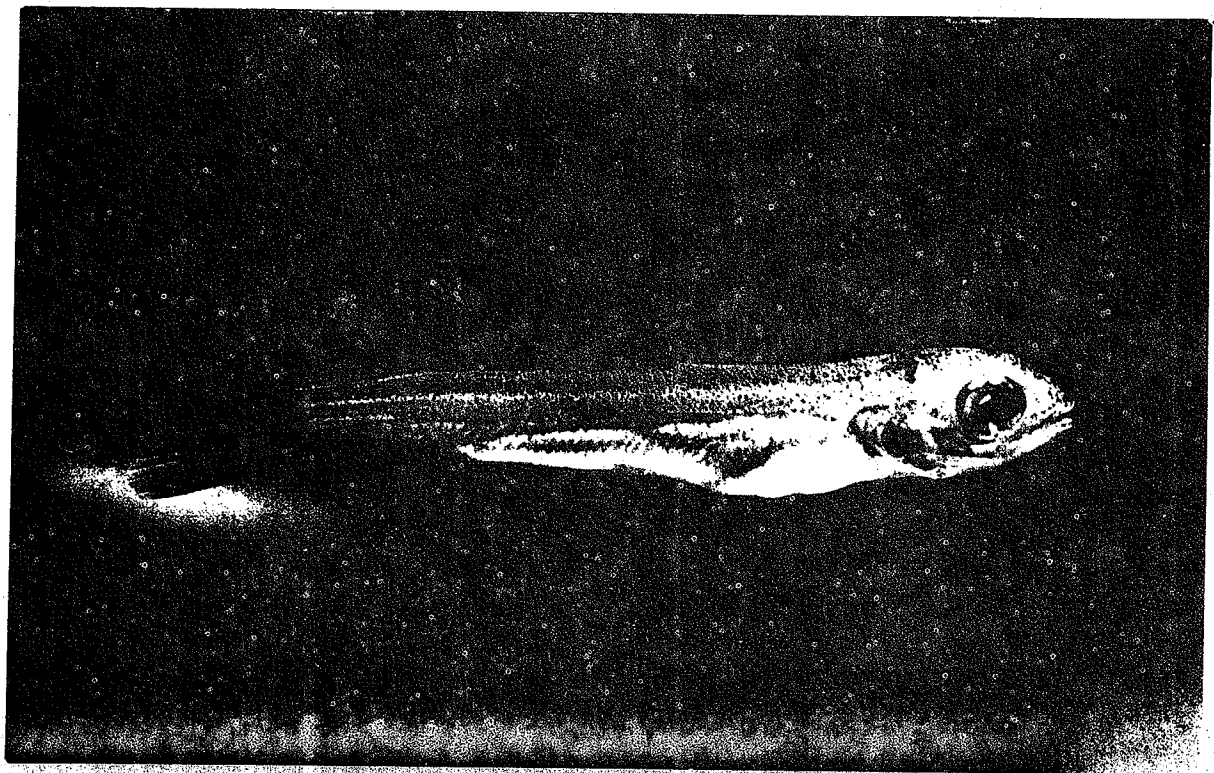
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To lake whitefish everywhere; they made it all possible.



Abstract

Genetic studies were undertaken to establish the bases of four electrophoretically polymorphic proteins in lake whitefish, Coregonus clupeaformis. These biochemical characters were then used to investigate zoogeographic problems in lake whitefish which had been posed as a result of morphological study by other workers.

Breeding experiments revealed the genetics of the electrophoretic phenotypes of glycerol-3-phosphate dehydrogenase (G-3-PDH) isozymes from white muscle. The G-3-PDH isozyme phenotypes were explained on the basis of a molecular, genetic model involving two loci, one having two alleles and the second three alleles. This model predicted, through simple Mendelian non-dominance, a total of eighteen phenotypes, fifteen of which were observed among 2200 lake whitefish from 38 lakes in Western Canada. Additional genetic information was derived from examination of lake whitefish muscle for phenotypes of lactate dehydrogenase (LDH) produced by a heart-type LDH locus. This locus is represented by two alleles, the genetics of which were determined in an earlier study (Clayton and Franzin, 1970). Less precise information was obtained from study of malate dehydrogenase (MDH) and hemoglobin phenotypes.

All biochemical data were used collectively to test an hypothesis, developed from morphological evidence, that lake whitefish existed in at least two refugia (Bering and Mississippi) during the Wisconsin glaciation, and that the variability seen among lake whitefish populations in Western Canada is at least partly due to a postglacial admixture of two or more discrete

stocks. The biochemical observations all revealed a break in gene frequencies at the periphery of the Yukon River watershed which is roughly consistent with morphological observations. Biochemical data suggest that gene flow in lake whitefish has been unidirectionally out of the Yukon River watershed. Possible postglacial routes of fishes originating from different glacial refugia are proposed on the basis of biochemical data combined with information on gillraker numbers and the distributions of other freshwater fish species. Local anomalies in gene frequencies, as compared with general patterns, apparently reflect combinations of founder effect, isolation, genetic drift and/or selection.

A tentative chronology, based on lake whitefish biochemical data and geological information, was devised to explain the post-glacial dispersal and distribution of lake whitefish and other freshwater fishes in the study area.

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INTRODUCTION

Students of evolution have long made use of morphometric techniques to detect differences within and between species and to document evolutionary trends and changes in organisms. The use of morphological features for such purposes is subject to two major shortcomings. First, the total extent of environmental influence on the development and final form of morphological features is rarely known. Secondly, generally little is known about the genes or numbers of genes controlling morphological features or their exact mode of inheritance.

The advent, in recent years, of biochemical techniques, particularly gel electrophoresis, has provided new and important tools for the evolutionary study of organisms. Electrophoresis, followed by histochemical staining, allows the separation and identification of single gene products. Through this technique, many proteins, particularly enzymes, have been found to exist in multiple forms both within individuals and species and have become useful as descriptive characters.

Biochemical characters offer some significant advantages over morphological characters. Electrophoresis separates the products of structural genes. Even though these products may be several regulatory steps removed from the actual genetic material, their identification is assured by a staining reaction which is tied specifically to the physiological role of a particular gene product. Identification of a single gene product allows genetic study of the

locus coding for that product through controlled breeding experiments. A few or several genetically independent biochemical characters can provide the researcher with a new powerful tool for the study of population structure, hybridization, introgression, etc., in a manner impossible by morphometric means. Biochemical characters are not without shortcomings. However, most problems can be avoided by abiding by three important precepts: 1) one must be aware of the extent to which expression of a biochemical character is influenced by the ontogeny of an organism, 2) one must be reasonably certain that identical electrophoretic phenotypes are produced by identical genes, and 3) one must be certain that he is dealing with a single species. These three precepts can be upheld by careful study of an organism including a controlled breeding experiment.

The development of the new field of biochemical population genetics has been the direct result of the application of biochemical methods to problems in evolution and zoogeography both within and among species of organisms. Studies such as those of Hunt and Selander (1973) on hybridization in European mice on the Jutland Peninsula of Denmark, Salthe (1969) on geographic variation in the frogs Rana pipiens and R. palustris in North America and the work of Koehn et al. (1971) on zoogeography and genetical population structure of the minnow, Notropis stramineus in the Kansas River system illustrate the kinds of information that may be gained through the use of biochemical methods. The combination of morphological information with that provided by biochemical techniques as accomplished by Hunt and Selander (1973) can be particularly

rewarding. These authors used biochemical techniques to study a zone of hybridization between two subspecies of house mice that had been defined on morphological grounds. They were able to demonstrate that the subspecies were capable of freely interbreeding, the extent and direction of introgression between them and the effects of a gradient of environmental factors on the hybrid zone. Thus biochemical study provided a considerable insight into a morphological problem.

North America offers an unique opportunity to study the effects on biological populations of a major environmental catastrophe in the form of the recent Wisconsin glaciation. During glaciation, the continent was effectively broken up into several disjunct refugia which probably differed one from another in terms of environmental and climatic factors. Organisms were essentially locked-up in these refugia for many thousands of years and then allowed with the demise of the glaciers, to once again intermix and reoccupy their former ranges.

The lake whitefish, Coregonus clupeaformis (Mitchill), is an example of a species severely affected by the Wisconsin glaciation, thus making it a good species through which to study the effects of the glaciation on fishes generally. Lake whitefish are ubiquitous and abundant throughout most of the area once covered by the ice sheets, commercially valuable and well-studied. However, because lake whitefish are morphologically extremely variable, their taxonomy has remained confused. Recent morphological work (Lindsey et al., 1970) has suggested that lake whitefish existed in at least

two refugia during glaciation with an associated development of slightly different morphological forms. Preliminary evidence from biochemical studies (Lindsey et al., 1970) tended to reinforce that suggestion.

The present study was motivated by the following questions:

1) Are biochemical genetic characters identified in lake whitefish suitable for use in zoogeographic study? 2) Does zoogeographic study of Western Canadian lake whitefish populations using biochemical characters provide support for hypotheses on the glacial and postglacial status of lake whitefish based on morphological observations? 3) What additional information to that from morphological studies, regarding postglacial dispersal, can be provided by using biochemical characters in a zoogeographic study of lake whitefish?

Following the section "Materials and Methods" detailing sample preparation, electrophoretic procedure, field collections and breeding experiments is a brief "Outline of Glacial History and the Zoogeographic Problem". The remainder of the thesis is divided into two discrete parts, each with its own results and discussion sections. The first part "Genetic Studies", concerns the genetic investigations of electrophoretic forms of four proteins: lactate dehydrogenase (LDH), glycerol-3-phosphate dehydrogenase (G-3-PDH), malate dehydrogenase (MDH) and hemoglobin. The second part, "Population Studies", considers the distribution and postglacial dispersal of lake whitefish in Western Canada based on population gene frequencies of those protein characters for which the genetics

were determined in the first section. These inferences are then compared to suggestions made from morphological study and study of the distributions of other species of freshwater fishes.

Initially it was hoped that lake whitefish populations from across Canada would have been included in this study but it was impossible to collect specimens from such an extensive distribution in the time available. Also, many eastern Canadian lake whitefish populations have been contaminated through multiple introduction of lake whitefish from the Great Lakes beginning in the late 1860's and continuing to the 1930's. Therefore, the present study was restricted to lake whitefish populations west of the Ontario-Manitoba boundary.

MATERIALS AND METHODS

Breeding Experiments

Information on the genetics of lake whitefish biochemical polymorphisms discussed in this study comes from two separate breeding experiments. The first, conducted during the author's M.Sc. research (Franzin, MS 1970), revealed the genetics of lake whitefish multiple lactate dehydrogenase (LDH) isozymes (Clayton and Franzin, 1970) and forms the basis of the present use of LDH gene frequencies in the study of lake whitefish populations. The second and larger breeding experiment, conducted in 1970-71, was carried out specifically for the clarification of glycerol-3-phosphate dehydrogenase (G-3-PDH) genetics and the elucidation of the genetics of hemoglobin and malate dehydrogenase (MDH) phenotypes. Some data on the inheritance of G-3-PDH phenotypes was also derived from the first breeding experiment. The data on G-3-PDH from both breeding experiments was combined in a paper published by Clayton et al., (1973).

All fish used in the 1970-71 breeding experiment were taken in Goose River (locally Rat Creek), the outlet from Lake Athapapuskow, Manitoba. Data from the 1968 breeding experiment which were used in the present study were derived from four matings (SII, SIII, SIV, SV) made with fish taken from Mistik Creek about 200 yards from its confluence with Lake Athapapuskow and two matings (GII, GV) made with fish from Goose River (Franzin, MS 1970). All these locations are shown in Figure 1.

Fertilization was effected by releasing eggs into a wet, shallow

Figure 1. Locations of spawn collection sites for both 1968
and 1970 breeding experiments.

