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

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

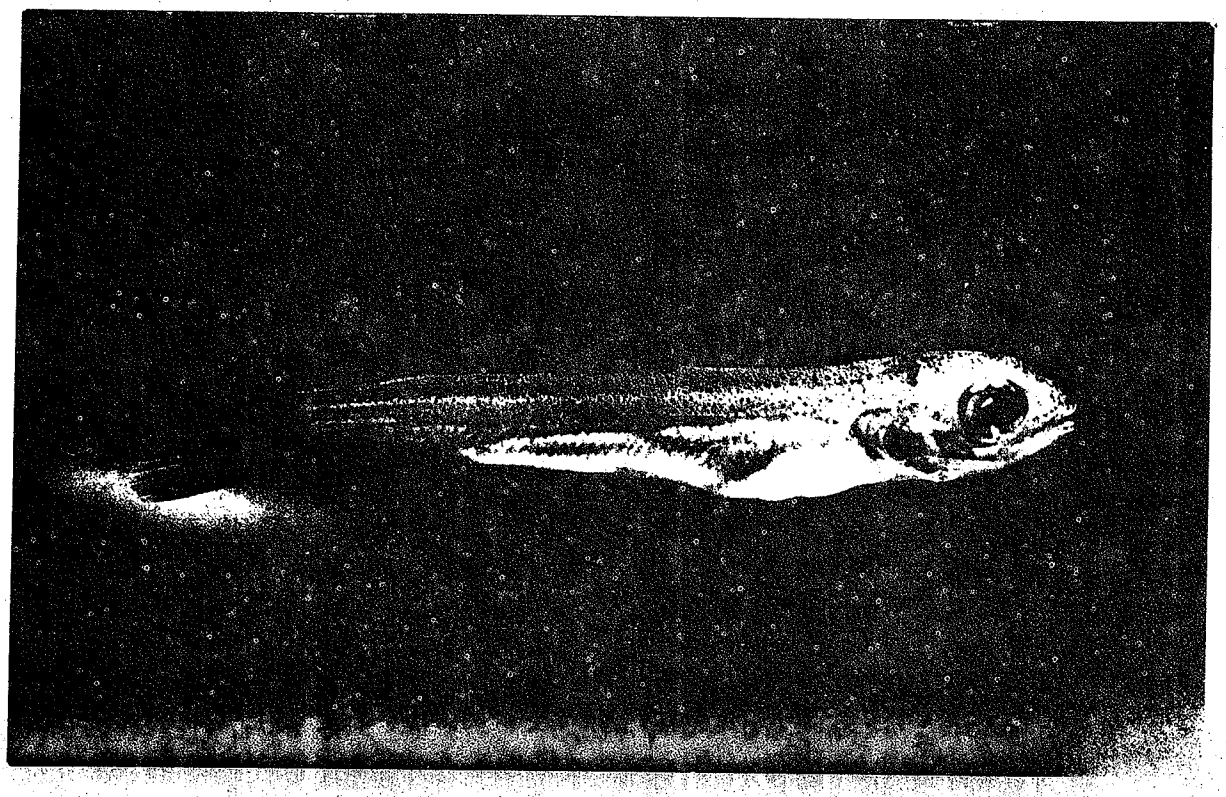
GENETIC STUDIES OF PROTEIN VARIANTS AND THEIR USE IN A ZOOGEOGRAPHIC STUDY OF LAKE WHITEFISH, COREGONUS CLUPEAFORMIS (MITCHILL) IN WESTERN CANADA

by

WILLIAM GILBERT FRANZIN

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY



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.

ACKNOWLEDGEMENTS

The author is indebted to his joint supervisors Drs. J.W. Clayton and C.C. Lindsey for assistance and advice as well as support in the laboratory and field. Thanks are due also to members of the Ph.D. Advisory Committee, Drs. G. Eales, D.E. McAllister, D.P. Scott and W. Woodbury. Advice and assistance of Dr. H.E. Welch, Chairman of the Zoology Department and other staff members in the department over the past several years was most appreciated. A special thanks is due my wife Shar. for help in the field, typing several drafts of the manuscript and moral support as required. Red Clarke, John Loch, Bob Manness and others also helped in field collecting while John Cook, Bob Harris, Bill Macdonald and Dan Tretiak provided additional help in the laboratory. Brian Billeck and Bob Gordon helped in both the field and the laboratory. Also, I wish to express my gratitude to the many people in Western Canada who helped to make the field collections possible. Advice and assistance with some basic computer manipulation of data was provided by Red Clarke. Dr. M. Healey deserves special thanks for provision of fish samples from lakes in the Yellowknife area. Mrs. G. Decterow is credited with typing of the final manuscript.

Financial support from the National Research Council and Fisheries Research Board, in grants to Dr. C.C. Lindsey, and from a National Research Council Scholarship to the author, is gratefully acknowledged. The Director and staff of the Fisheries Research Board, Environment Canada, Freshwater Institute, are thanked for provision of laboratory space and materials as well as many other services.

TABLE OF CONTENTS

Table of Contents (concluded)

LIST OF TABLES

LIST OF FIGURES

List of Figures (cont'd)

List of Figures (cont'd)

LIST OF APPENDIX FIGURES

List of Appendix Figures (cont'd)

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

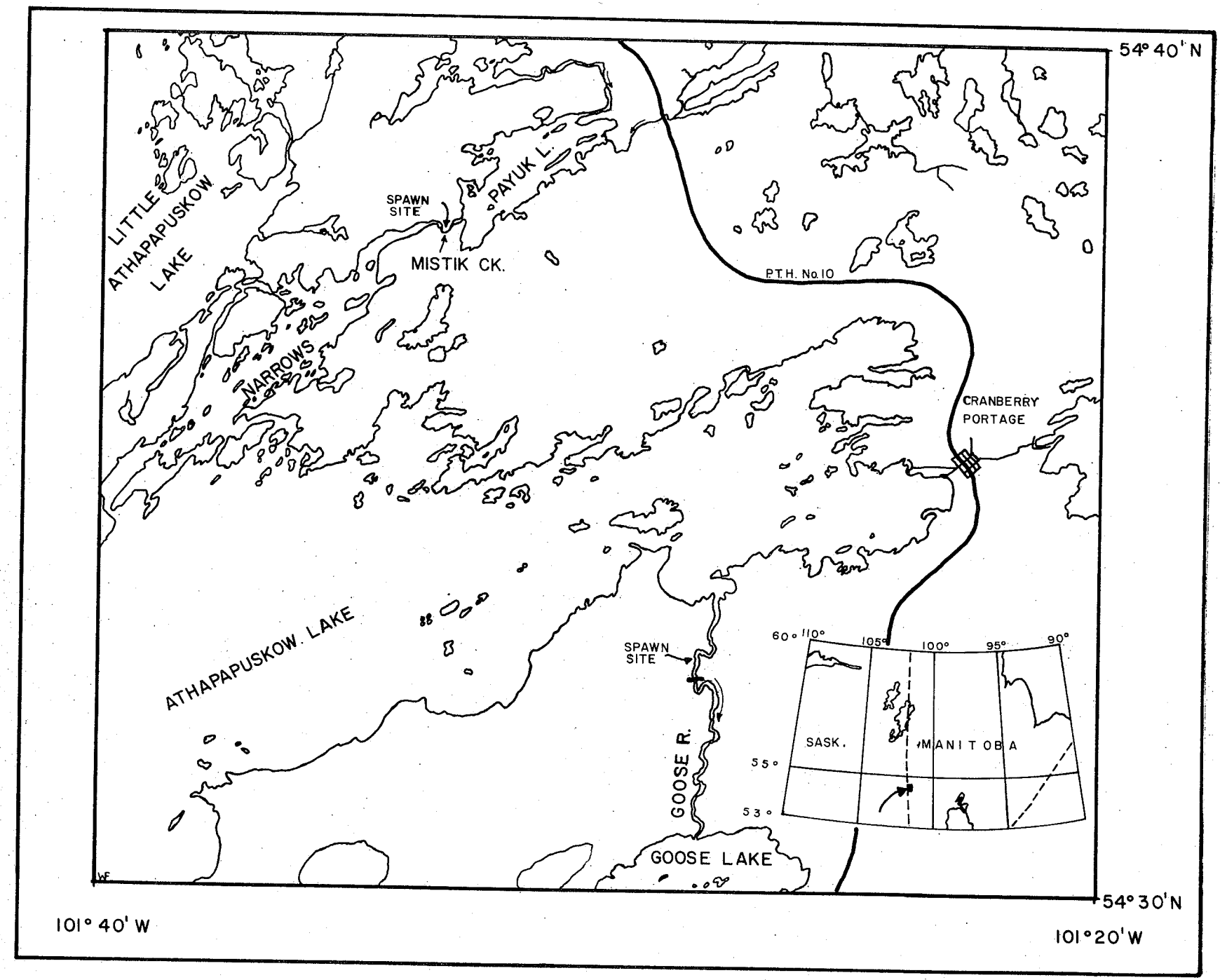
Breeding Experiments

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.


54° 40' N
LITTLE ATHAPAPUSKOW LAKE
SPAWN SITE
MISTIK CK.
PAYUK L.
P.T.H. No. 10
NARROWS
CRANBERRY PORTAGE
ATHAPAPUSKOW LAKE
SPAWN SITE
GOOSE R.
GOOSE LAKE
60° 110° 105° 100° 95° 90°
SASK.
MANITOBA
55°
53°
54° 30' N
101° 40' W
101° 20' W

bowl and then mixing sperm into them. After a few minutes the eggs were rinsed with water and transferred to marked screened baskets made from commercial one pint plastic freezer containers. The baskets were kept in water-filled styrofoam coolers with continuous aeration while in the field. For transport to the laboratory the baskets were transferred to large plastic bags about half-filled with oxygenated lake water, topped with gaseous oxygen, sealed and packed in ice within styrofoam coolers. Spent fish were killed, bled and the carcasses and blood samples iced and transported to the Freshwater Institute in Winnipeg for biochemical analyses. To this point both breeding experiments were similar. Details of subsequent egg handling in the 1968 experiment are reported elsewhere (Franzin, MS 1970, Clayton and Franzin, 1970) and will not be discussed further. Subsequent discussion here refers to the 1970-71 breeding experiment.

Incubation of fertilized eggs from 29 matings obtained from 9 female and 18 male lake whitefish on October 24 and 25, 1970 was conducted initially in the Animal Holding Facility of the Zoology Department, University of Manitoba. Soon after their arrival in the Zoology Department, the eggs were transferred to shallow, stainless-steel, screened trays hung just below the surface in flowing water at 4-5 C. Eggs from each mating were maintained in separate tanks or screened portions of water troughs. On November 12, 1970, a portion of each of 20 of the individual egg lots was transferred to the Freshwater Institute. The incubation of these eggs was conducted in flowing tap water in small hatchery jars equipped

with inlet pipes that extended to the bottom of each jar, thus effectively suspending the eggs in a gentle upward flowing current of water. The water in this system was maintained by refrigeration at 1-2 C, and treated with sodium thiosulphate to neutralize any free chlorine.

Hatching, in the egg lots maintained in the Zoology Department at 4-5 C, occurred in February, 1971 while egg lots incubated in the Freshwater Institute, at the more natural temperatures of 1-2 C, hatched in April, 1971. Egg mortality varied among batches incubated in the warmer conditions but was greatly reduced among lots incubated in colder water. No obvious differential mortality was noticed within either group of egg lots. After hatching was completed the water temperature in rearing tanks was slowly raised (approximately 1 C/day) to 12-16 C and maintained in this range for the remainder of rearing. Young fish were fed a mixed diet of live brine shrimp nauplii, finely ground EWOS salmon starter or trout food and live plankton from local ponds. By August, 1971 the young whitefish had reached a usable size (4-10 cm) and analyses were begun. The last of the fish were killed by September 2, 1971.

Field Collections

Collections of lake whitefish were obtained by gillnetting during the period 1967-1972 by the author, co-workers and others. Fish carcasses were air shipped or trucked on ice within 48 hours of capture, to the Freshwater Institute in Winnipeg where they were immediately deep frozen and stored. Some fish were frozen and stored for a short period at different places prior to shipment to Winnipeg. Blood, when

taken, was iced and shipped to the laboratory within 48 hours of sampling. Carcasses were sampled for muscle tissue after periods of cold storage varying from a few weeks to a few years. There was no indication that any of the characters was influenced by long term cold storage.

Biochemical Analyses

Four protein characters were used in this study: the three enzymes lactate dehydrogenase, malate dehydrogenase and glycerol-3-phosphate dehydrogenase, and hemoglobin.

Analytical methods used were as follows.

Lactate Dehydrogenase (LDH)

The methods used for analysis of lake whitefish red muscle tissue extracts for LDH activity were those described by Clayton and Franzin (1970).

Malate Dehydrogenase (MDH)

Analyses for MDH activity in extracts of lake whitefish muscle tissue were carried out as described for walleye by Clayton et al. (1971) except for the following changes which were made to clarify the lake whitefish electrophoretic phenotypes:

1) Tissue samples from both adult and juvenile lake whitefish were macerated in a 1:3 ratio (by weight) of tissue to the 300 mg/l solution of nicotinamide adenine dinucleotide (NAD).
2) The head, viscera and backbone of juvenile lake whitefish were removed prior to maceration of the remaining tissues.

3) The electrophoresis buffer was N-(3-aminopropyl) morpholine-citrate, pH 6:1 at 22 C, as described by Clayton and Tretiak (1972). NAD was added to both gel and bridge buffers to a concentration of 100 mg/l.

Glycerol-3-Phosphate Dehydrogenase (G-3-PDH)

Methods used for analysis of lake whitefish muscle tissue extracts for G-3-PDH activity were reported in Clayton et al. (1973).

Hemoglobin

1) Adult Fish: Adult fish were sampled alive or within a few hours of death by puncture of caudal blood vessels just posterior to the anal fin. Vacutainer^{R} (Becton Dickinson Company) blood collecting equipment consisting of a 7 ml draw heparinized vacuum tube fitted with an adapter and a double-ended 1 1/2" 20 guage needle was used for this purpose. Blood was allowed to fill to a maximum of half the tube then diluted with an equal volume of 1% NaCl solution. The tube was agitated, then stored on ice for transport to the laboratory within 48 hours.

2) Progeny: Young fish were killed in a lethal solution of MS 222[1]. Within a few minutes of death the peduncle was severed and blood was collected in heparinized capillary tubes. The blood was then transferred to disposable microcentrifuge tubes containing a few drops of 1% NaCl solution.

---

[1] Tricaine methanesulphonate, Kent Labs. Ltd., Vancouver, B.C.

3) Both adult and juvenile fish blood was handled similarly from this point with respect to differences in sample size between adults and juveniles. Red blood cells were washed in carbon monoxide saturated 1% NaCl and centrifuged several times until the supernatant above the cells was clear. Hemolysis was effected in one hour by suspending the cells in double their volume of carbon monoxide saturated distilled water. The hemolysate was centrifuged to remove cell debris and the translucent red hemoglobin solution was used directly for application to starch gel supports.

4) Electrophoresis: Electrophoresis was carried out as described by Tsuyuki et al. (1966). Starch gels were made by boiling 10% by weight of Connaught hydrolyzed starch in 120 ml of 0.023 M boric acid adjusted to pH 8.5 with NaOH. Bridge buffers were 0.030M boric acid also adjusted to pH 8.5 with NaOH. Both buffers contained 0.025% EDTA[2]. Electrophoresis was carried out as for LDH. Gels were stained for 15 minutes in a 5:1:5 solution by volume of methanol, acetic acid and water containing 0.1% amido black 10 B. Prior to storage, the gels were washed free of excess background stain by several washes with the 5:1:5 solution of methanol, acetic acid and water.

---

[2] EDTA: Ethylene diamine tetraacetic acid.

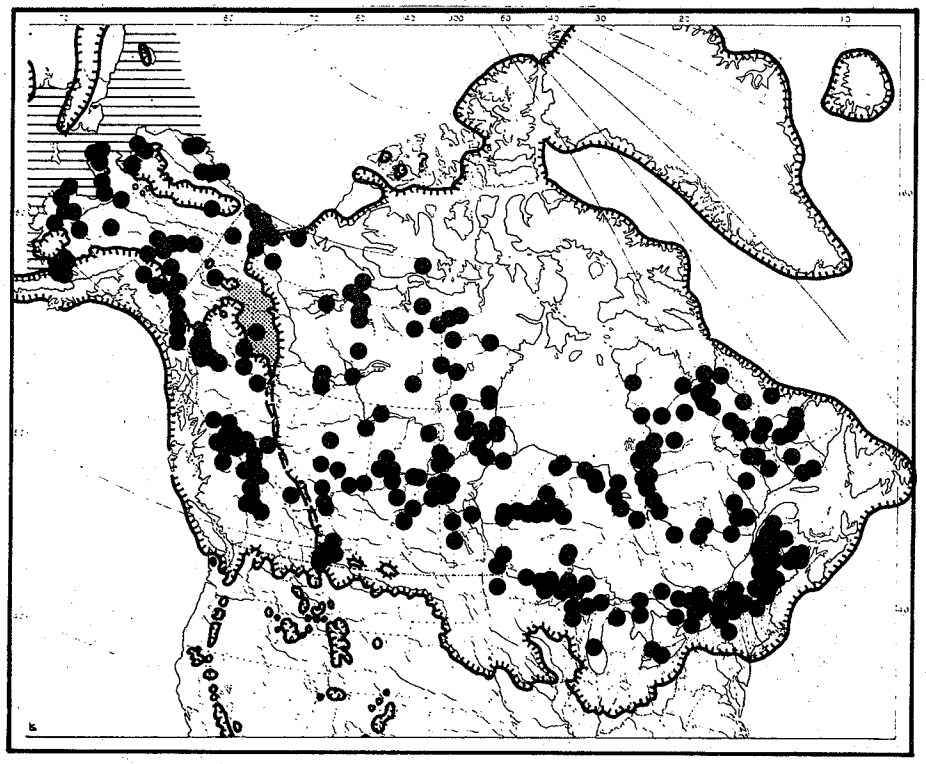
Outline of Glacial History and the Zoogeographic Problem

Lake whitefish are presently distributed over most of northern North America (Figure 2) principally within the area once covered by the vast Wisconsin ice sheets. From which glacial refugium are the bulk of Canadian lake whitefish populations derived and by what routes did this species colonize the area vacated by the vanished Wisconsin glaciers? An appreciation of this problem is enhanced by an overview of the events of glaciation and deglaciation.

There have been four major glacial periods over the last one million years, each lasting about one hundred thousand years and separated by longer interglacial periods. The last of these, the Wisconsin, is the only glacial period for which detailed information is available and is the one that interests us here.

The Wisconsin glaciation reached its maximum about 20,000 years ago and by about 7,000 years ago the major ice sheets had disappeared. Glaciation began in three centers--two in the east, in Labrador and in Keewatin around the western side of Hudson Bay, and one in the west, a Cordilleran complex which arose in the Coast Mountains and related high areas of the interior of northern British Columbia and southern Yukon. As the developing glaciers thickened, they began flowing out from these centers in a radiating pattern governed more or less by topographic features. By ice maximum, the Cordilleran and Laurentide (Keewatin and Labrador lobes together) ice sheets abutted each other over most of the length of the Canadian Rocky Mountains. It was during this period

Figure 2. The distribution of the lake whitefish, Coregonus clupeaformis in relation to Wisconsin glaciation (From Lindsey et al., 1970).



that fishes as well as other organisms were confined to the refugia shown in Figure 3. Also during this time, until about 11,000 B.P. (Before Present) (Walters, 1955), the Bering land bridge emerged due to a drop of a few hundred feet in the level of the seas.

Deglaciation proceeded roughly in the reverse of glaciation. Ice began receding from southwestern Alberta around 14,000 B.P. and the ice margin pulled back rather uniformly northeastward in a northwest-southeast line. By about 10,000 B.P. the ice margin was well back from the Rocky Mountains, probably opening up the area west of a line drawn roughly from around the Mackenzie River Delta to Great Slave Lake to Lake Athabasca to about the middle of what is now Lake Winnipeg. This was a time of huge proglacial lakes (in which ice formed one boundary) (Figure 4). Restricting this discussion to west of the Ontario-Manitoba boundary, one of the major proglacial lakes was Lake Agassiz which covered much of southern Manitoba and adjacent U.S. states from about 12,000-8,000 B.P. This lake went through a series of levels during its 4,000 year history, sometimes draining south, east or northwest and sometimes more than one way at the same time. Elson (1967) gives a detailed history of Lake Agassiz. During this same time, the Great Lakes also went through a complex history of levels and outlets; these are described by Prest (1970).

To the west, in central Alberta, a series of lakes in the Edson-Edmonton area is described by St. Onge (1972). Just north of there, a large glacial Lake Peace, collecting waters from the

Figure 3. Maximum extent of glaciation during the Wisconsin period. Arrows suggest directions of ice advance. Letters indicate refugia for freshwater fishes: A = Alaska (Bering), B = Pacific, C_1 = Missouri River, C_2 = Mississippi River, E = Exposed Bering land bridge, T = Atlantic (From McPhail and Lindsey, 1970).

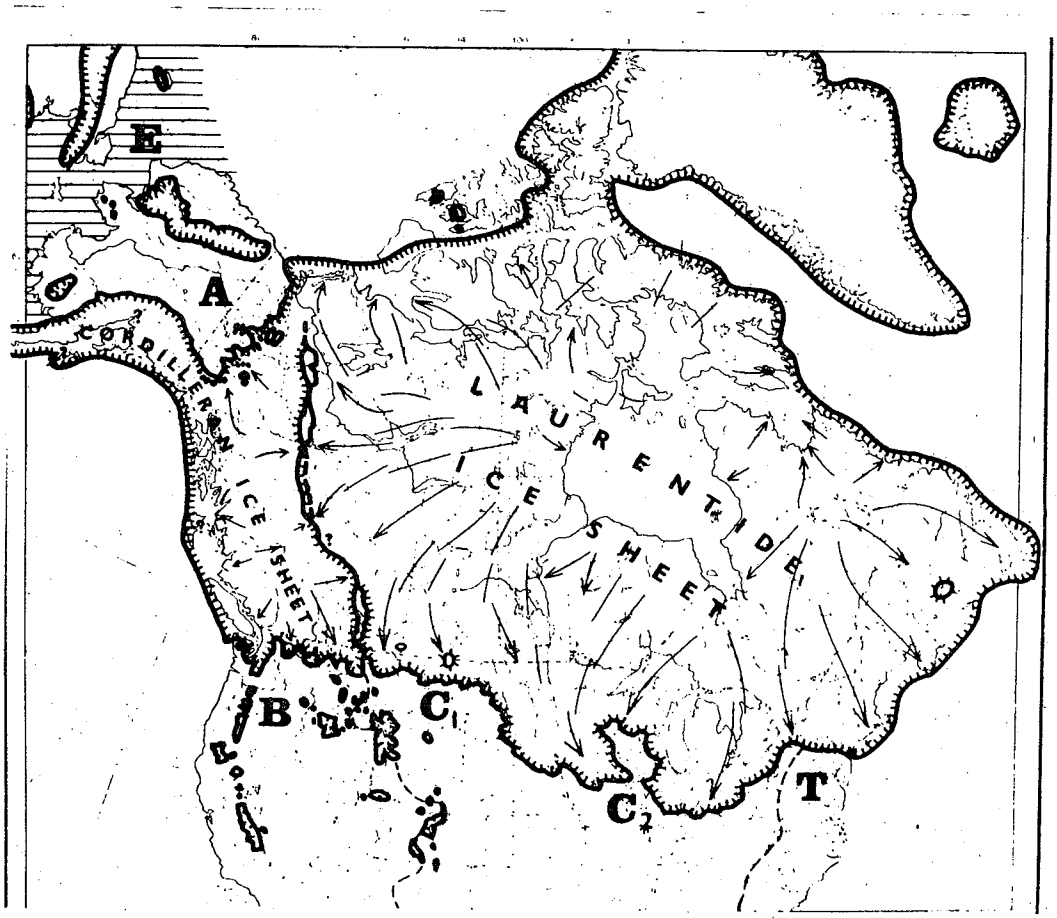
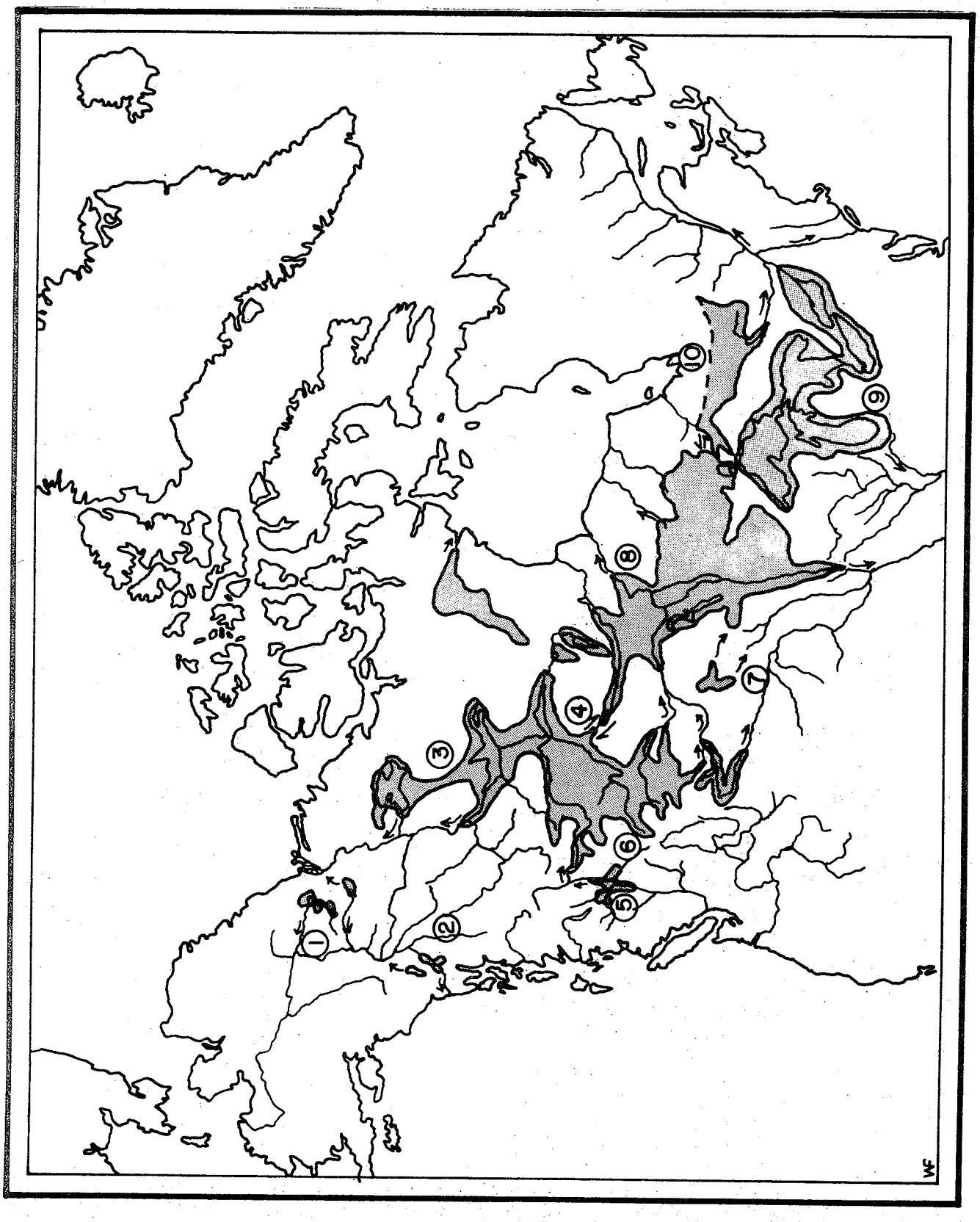

E
A
CORDILLERAN ICE SHEET
LAURENTIDE ICE SHEET
B
C1
C2
T

Figure 4. Schematic representation of maximum limits of glacial lakes in Canada; not necessarily contemporaneous. 1) Lakes in the unglaciated area and Bonnet Plume Basin (Hughes, 1972), 2) Glacial Lakes Champagne, Kloo, Fisher and Alsek (some at different times in the same location) (Kindle, 1953), 3) Glacial Lake McConnell (Craig, 1965), 4) Glacial Lake Tyrrell (Taylor, 1960), 5) Glacial Lake Prince George (Tipper, 1971), 6) Glacial Lake Peace (Taylor, 1960) and the lake series in the Edmonton area (St. Onge, 1972), 7) Glacial Lake Regina (Prest, 1970), 8) Glacial Lake Agassiz (Elson, 1967), 9) Glacial Great Lakes (Prest, 1970), 10) Glacial Lake Barlow-Ojibway (Prest, 1970).


1
2
3
4
5
6
7
8
9
10
WF

central interior of B.C., first drained southeastward to Lake Agassiz but later changed to a northward drainage to the Mackenzie River (Taylor, 1960). In southern Alberta, smaller ephemeral lakes drained first into the Missouri system but finally changed to a northeastward drainage via South Saskatchewan River into Lake Agassiz. Similarly in southern Saskatchewan small glacial lakes first drained via the Souris River into the Mississippi River but later into Lake Agassiz via Assiniboine and Qu'appelle rivers.

In the far north giant glacial lakes formed--Lake Tyrrell (a final northern stage of Lake Peace) lying in the western part of the Lake Athabasca Basin was succeeded by Lake McConnell which covered the basins of Great Bear, Great Slave and Athabasca lakes plus the intervening areas (Craig, 1965). These lakes early drained southeastward to Lake Agassiz but later the flow was reversed and for a short time Lake Agassiz may have drained northwestward (Elson, 1967). As the land rose up from the removal of the weight of the ice sheet this watercourse dried up; the northern lakes began a northward drainage into the Arctic Ocean and Lake Agassiz resumed a southern drainage.

West of the Rocky Mountains, the Fraser River remained ice-dammed in its middle reaches for some time, causing a large lake to form in the region of Prince George and another small one just to the west, in the Nechako River Valley. These lakes drained northward via the Parsnip River into the Peace River which carried these waters into Lake Peace. Thus waters from

glaciers on the Coast Mountains, via the chain of glacial lakes outlined, probably reached the Gulf of Mexico and perhaps the Atlantic Ocean (via Lake Agassiz-Great Lakes connections) (Prest, 1970).

Glacial lakes were numerous also in the Yukon--one large lake was Glacial Lake Champagne which occupied a portion of the Yukon River Valley and was connected to the valley of the Alsek River, presently a Pacific coast glacial stream. Other lakes were present in the valleys of many Yukon River tributaries but drainage connections made by them were within the Yukon River system. Waters were exchanged perhaps as many as four times between the Yukon River drainage and the Peel River of the lower Mackenzie River system through the action of ice-dammed lakes (Hughes, 1972; Pers. Comm.). In the mountainous areas of B.C. and Yukon, headwater exchanges were probably common due to local glacial action.

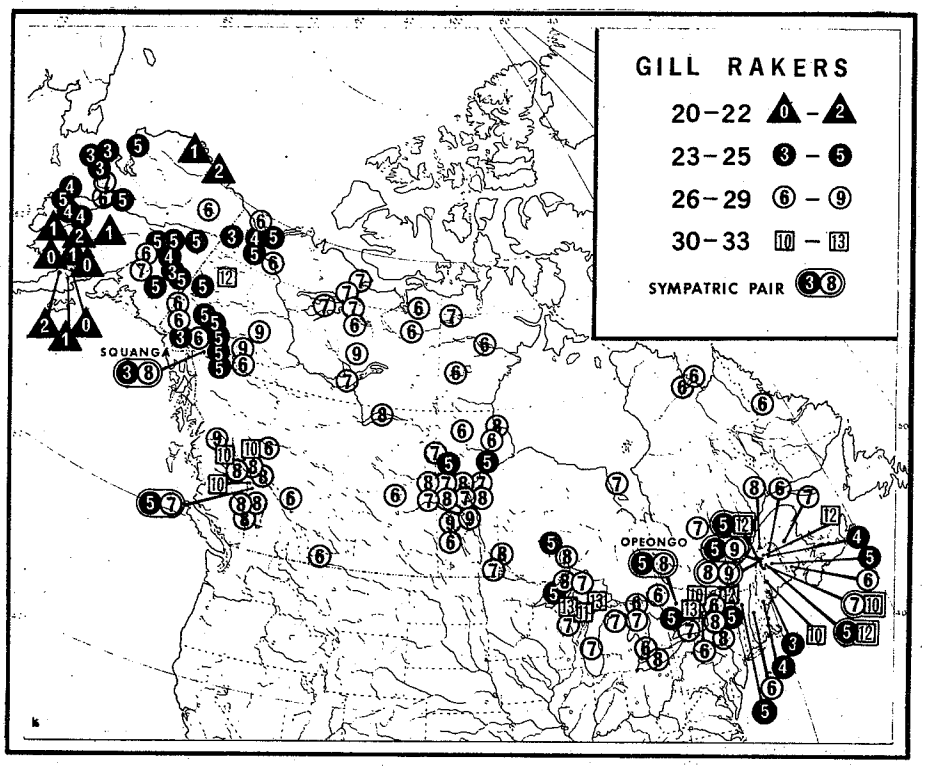
A more detailed account of Wisconsin glaciation and deglaciation can be found in Appendix 1.

Upon this historically fluctuating and discontinuous environment of northern North America, the lake whitefish distribution is now superimposed. It appears likely, from geological studies of Wisconsin proglacial lakes and watercourses, that avenues of dispersal for lake whitefish from two of their proposed major refugia (Lindsay et al., 1970) were well provided. The long-standing glacial Lake Agassiz must certainly have provided a significant route for the dispersal of lake whitefish from a Mississippi refugium into much of eastern central and northwestern Canada.

Geological evidence for possible dispersal routes for lake whitefish emigrating from a Bering refugium is not as clear cut; but it is probable that many watercourses in the mountainous areas surrounding that refugium have changed directions at various times due to local glacial action, thus providing some means of dispersal from the Bering refugium into central Canada. The zoogeographer is presented with the challenging problems of defining from where and by what routes did lake whitefish come to occupy their present distribution.

Early work on this problem centered on the use of the number of gillrakers on the first gill arch to characterize lake whitefish populations by a mode of gillraker number. These structures are reputed to be of polygenic inheritance (as one would expect of a quantitative character) and Svärdson (1952) showed for one European species of whitefish that the gillraker number of progeny from a single mating reared under the same conditions varied only plus or minus two about the mean. The distribution of modal gillraker numbers for populations of North American lake whitefish seemed to indicate a trend toward lower numbers in the northwest with higher counts in the south and east. However, more evident was a rough break between Yukon populations and those of central Canada and B.C. (Figure 5) (Lindsey et al., 1970). Thus the distribution of modal gillraker numbers in lake whitefish populations gave some idea of discontinuity in the distribution of the species but it seemed desirable to find some other means by which to describe lake whitefish populations.

Figure 5. Distribution of population modes of gillraker number (the first arch, left side) in lake whitefish in North America. Paired numbers denote lakes containing two populations at least partially discrete in terms of gillraker number (From Lindsey et al., 1970).


GILL RAKERS
20–22 0 – 2
23–25 3 – 5
26–29 6 – 9
30–33 10 – 13
SYMPATRIC PAIR 3 8
SQUANGA
OPEONGO

Tsuyuki (1966) showed that lake whitefish exhibited more than one electrophoretic type of hemoglobin and in the late 1960's it became apparent, through work in the laboratory of Dr. J.W. Clayton that lake whitefish, like many other animals, had several electrophoretic forms of some proteins. Further, similar frequencies of electrophoretic phenotypes of some of these proteins in different wild populations of lake whitefish seemed to indicate no obvious environmental influence on them. Therefore it was logical to suppose that these electrophoretic protein phenotypes in lake whitefish had relatively simple genetic bases and could be useful for describing genetic relationships among populations as has been demonstrated for variants of hemoglobin and some enzymes in humans and other mammals. Having genetic information about specific loci of lake whitefish would be a step forward from knowledge based on morphological characters; perhaps then the relationships of present lake whitefish populations to the different glacial refugia would be clearer.

RESULTS AND DISCUSSION

I. Genetic Studies

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme which catalyzes the reaction, lactate + $NAD^+ \rightleftharpoons$ pyruvate + NADH + H^+ (White et al., 1964), an important mechanism in the maintenance of cellular redox balance. In most vertebrates two genes code for LDH subunits in muscle tissues; an m gene is active mainly in skeletal muscle while in heart muscle the activity of an h gene predominates. Some vertebrates produce only M_4 and H_4 tetramers while in others the two types of subunits may combine to produce three or five tetrameric isozymes.

Clayton and Franzin (1970) found that electropherograms of lake whitefish muscle extracts, as had been demonstrated in other salmonid fishes (Klose et al., 1968; Ohno et al., 1968; Bailey and Wilson, 1968; Massaro and Markert, 1968), revealed more LDH isozyme bands than most other vertebrates. Electropherograms of lake whitefish red muscle extracts revealed LDH activity as two discrete sets of isozymes, one typical of skeletal muscle and the other typical of heart muscle. These sets of isozymes were explained on the basis that lake whitefish possessed two loci for each of the M and H type LDH subunits. This was consistent with the hypothesis that salmonid fishes have undergone tetraploidization in their evolution (Ohno and Atkin, 1966; Ohno et al., 1968; Klose et al., 1968; Ohno et al., 1969).

Clayton and Franzin (1970) described three electrophoretic phenotypes of heart type LDH found in wild populations of lake whitefish (Figure 6). They proposed a model on a genetic and molecular basis to explain the observed phenotypes (Figure 6). The model proposed the existence of two non-dominant alleles $\underline{h}^b$ and $\underline{h}^c$ at the LDH locus coding for the electrophoretically most anodal heart type subunits. Thus the three phenotypes, designated FF, FS and SS according to electrophoretic migration were produced by genotypes $\underline{h}^a\underline{h}^a/\underline{h}^c\underline{h}^c$, $\underline{h}^a\underline{h}^a/\underline{h}^b\underline{h}^c$ and $\underline{h}^a\underline{h}^a/\underline{h}^b\underline{h}^b$ respectively. All fish possessed the same genotypes $\underline{h}^a\underline{h}^a$ for the least anodal heart type LDH locus. The asymmetry of the FS and SS phenotypes was believed to be the result of nearly coincident electrophoretic mobility of subunits coded for by the $\underline{h}^a\underline{h}^a$ locus and the $\underline{h}^b$ allele of the more anodal locus. Extensive electrophoretic tests over a range of pH did not resolve the expected isozymes. Superposition of LDH isozymes has been demonstrated in trout using immunological methods (Bailey and Wilson, 1968; Holmes and Markert, 1969). Possibly these methods or simple densitometric studies of relative staining intensities of isozymes such as those employed by Bailey et al. (1970) would yield evidence to support the contention that asymmetry of lake whitefish LDH phenotypes is due to superposition of isozymes.

A breeding experiment was carried out to confirm the genetic model for lake whitefish LDH. Although matings involving all combinations of the three phenotypes would have been desirable, only two types of matings, FS x SS and SS x SS, were obtained. However, the uniform fit of the experimental results obtained (Table 1) to

Figure 6. Model of lake whitefish heart-type LDH phenotypes showing presumed subunit composition of isozyme bands. Electrophoretic conditions outlined in Materials and Methods.

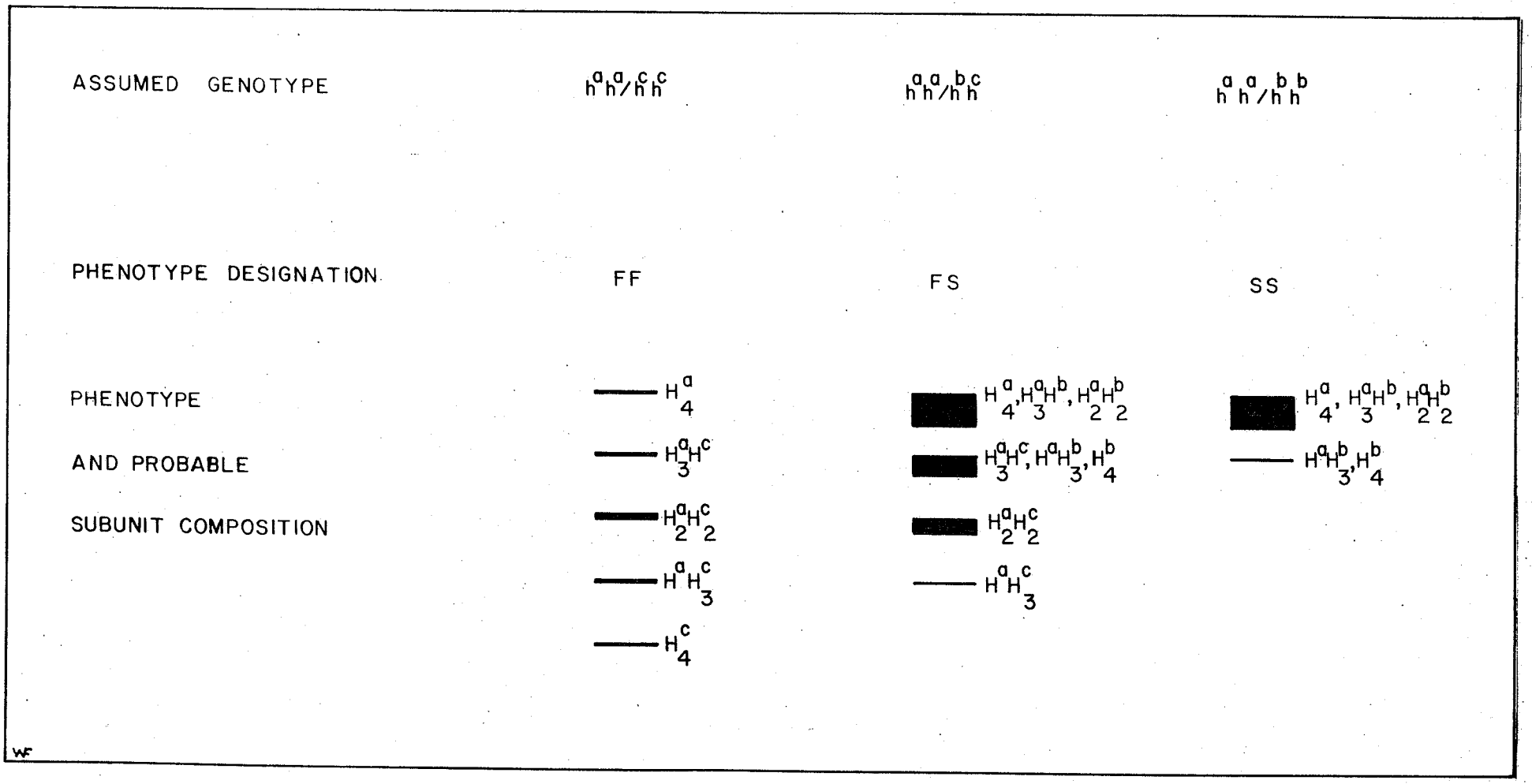

ASSUMED GENOTYPE
$h^a h^a / h^c h^c$
$h^a h^a / h^b h^c$
$h^a h^a / h^b h^b$
PHENOTYPE DESIGNATION
FF
FS
SS
PHENOTYPE
AND PROBABLE
SUBUNIT COMPOSITION
H_4^a
$H_3^a H^c$
$H_2^a H_2^c$
$H^a H_3^c$
H_4^c
$H_4^a, H_3^a H^b, H_2^a H_2^b$
$H_3^a H^c, H^a H_3^b, H_4^b$
$H_2^a H_2^c$
$H^a H_3^c$
$H_4^a, H_3^a H^b, H_2^a H_2^b$
$H^a H_3^b, H_4^b$

Table 1. Inheritance of lactate dehydrogenase in lake whitefish red muscle (from Clayton and Franzin, 1970).

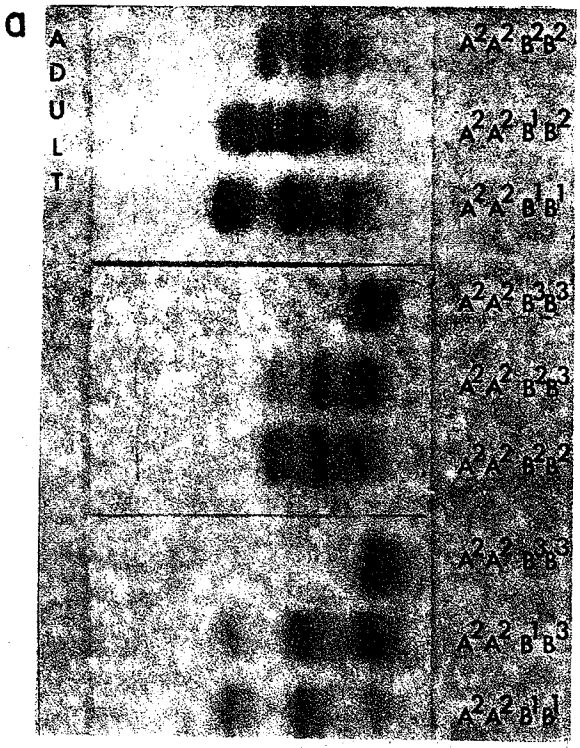
Mating	Parental Phenotypes		No. of progeny	
	Male	Female	FS	SS
1	SS	SS	0	24
2	SS	SS	0	36
3	SS	FS	26	15
4	SS	FS	34	38
5	SS	FS	18	18
Total	SS	FS	78	71

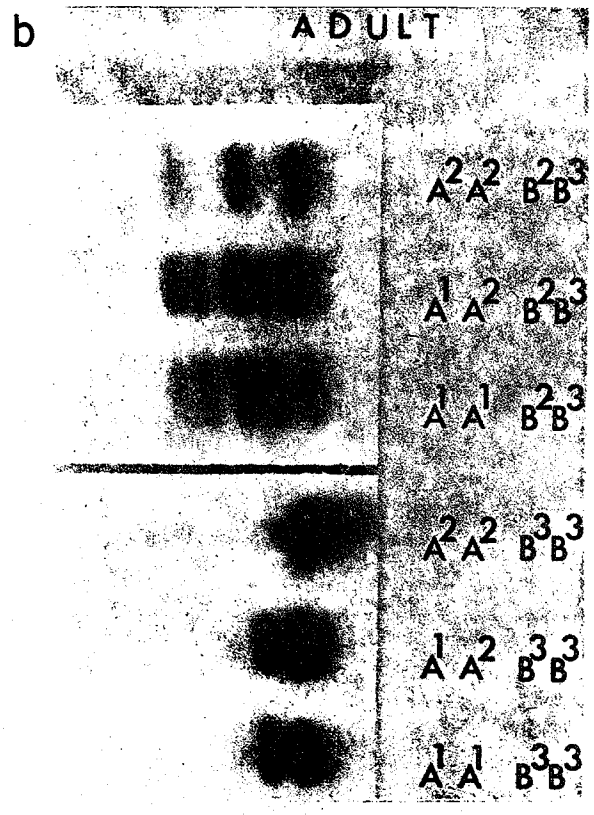
those predicted on the basis of the model, coupled with the fit of observed frequencies of the phenotypes in wild populations to phenotypic frequencies predicted by the Hardy-Weinberg equilibrium, provided adequate grounds on which to establish the genetics of the observed phenotypes. Although the data presented by Clayton and Franzin (1970) supported the hypothesis of a tetraploid event in the history of salmonids, genetically, in terms of the most anodal heart type LDH locus, lake whitefish behave like double diploid organisms.

Glycerol-3-Phosphate Dehydrogenase (G-3-PDH)

Glycerol-3-phosphate dehydrogenase catalyzes the reaction: dihydroxyacetone phosphate + NADH + H^+ $\rightleftharpoons$ glycerol-3-phosphate + NAD^+ (White et al., 1964). The enzyme is well known, and in its active state consists of a dimer (Broesmer and Marquardt, 1966, Marquardt and Broesmer, 1966, Van Eys et al., 1964). Starch gel electrophoresis of white muscle extracts from lake whitefish, followed by specific staining for G-3-PDH activity revealed an extraordinary number of phenotypes in wild populations. The phenotypes varied in both number and mobility of isozyme bands present (Figure 7 a, b, c). A model, (Figure 8) to explain the observed phenotypes, was developed on a combined genetic and molecular basis, assuming that whitefish G-3-PDH existed as a dimer in its active state. The model also assumes two genetic loci, a and b, to be active in lake whitefish white muscle, and predicts the formation of all possible types of AA, AB and BB dimeric isozymes. Further, the model proposes

Figure 7(a,b,c) Electropherograms of G-3-PDH isozymes in white muscle of adult lake whitefish. Electrophoretic conditions outlined in Materials and Methods.


a
ADULT
$A^2A^2\ B^2B^2$
$A^2A^2\ B^1B^2$
$A^2A^2\ B^1B^1$
$A^2A^2\ B^3B^3$
$A^2A^2\ B^2B^3$
$A^2A^2\ B^2B^2$
$A^2A^2\ B^3B^3$
$A^2A^2\ B^1B^3$
$A^2A^2\ B^1B^1$


b
ADULT
$A^2A^2\ B^2B^3$
$A^1A^2\ B^2B^3$
$A^1A^1\ B^2B^3$
$A^2A^2\ B^3B^3$
$A^1A^2\ B^3B^3$
$A^1A^1\ B^3B^3$

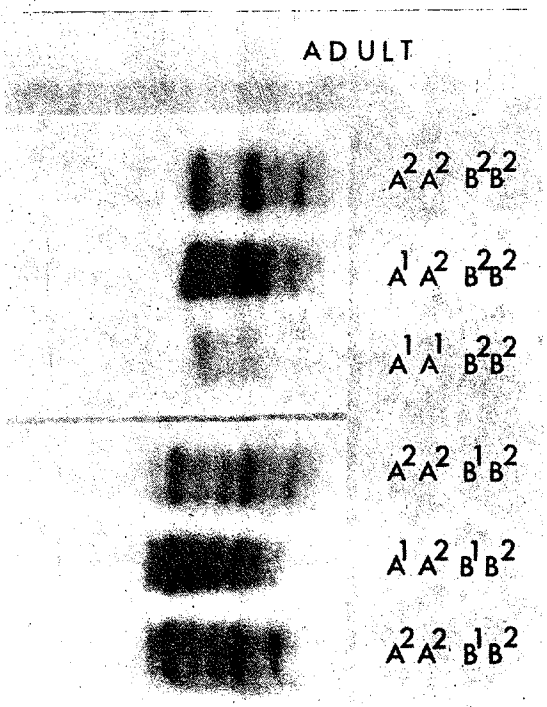
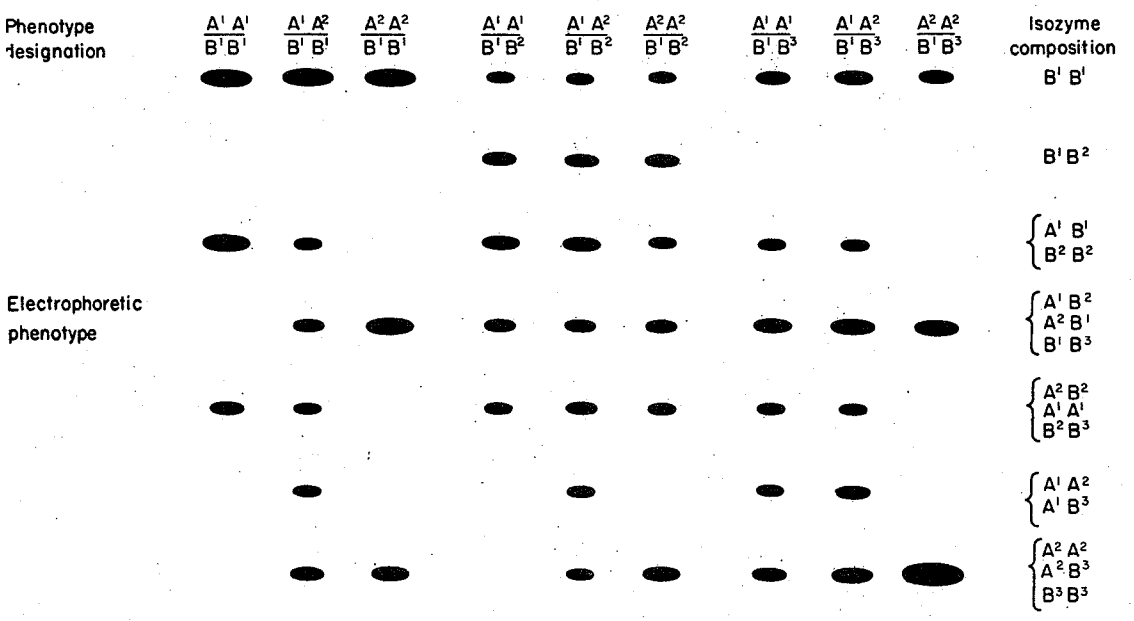
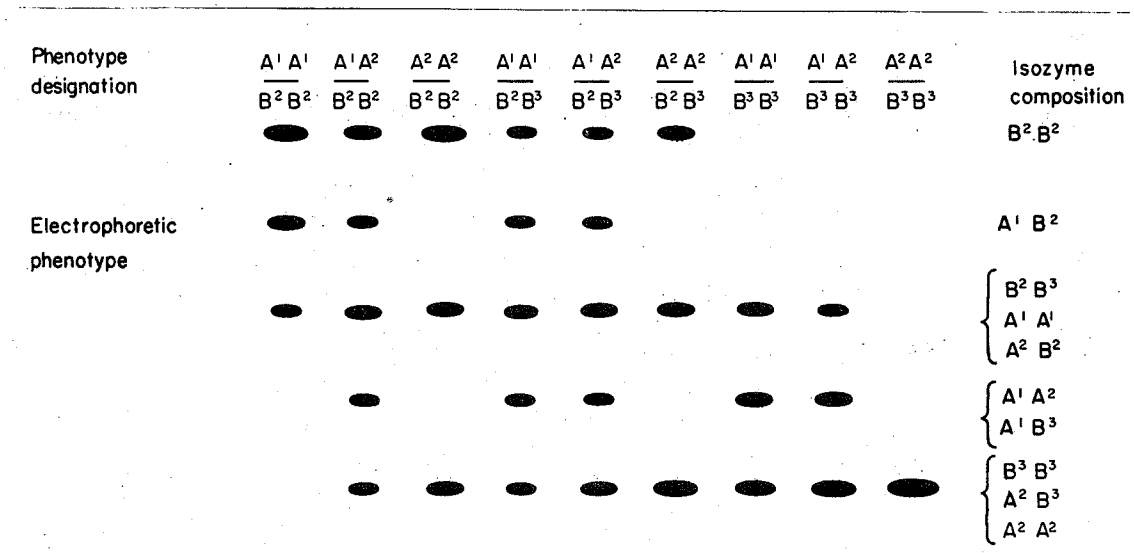

c
ADULT
$A^2A^2\ B^2B^2$
$A^1A^2\ B^2B^2$
$A^1A^1\ B^2B^2$
$A^2A^2\ B^1B^2$
$A^1A^2\ B^1B^2$
$A^2A^2\ B^1B^2$

Figure 8 Diagram of lake whitefish white muscle G-3-PDH phenotypes showing assumed subunit composition of the isozymes. Electrophoretic conditions outlined in Materials and Methods.


Phenotype designation
A¹A¹/B¹B¹
A¹A²/B¹B¹
A²A²/B¹B¹
A¹A¹/B¹B²
A¹A²/B¹B²
A²A²/B¹B²
A¹A¹/B¹B³
A¹A²/B¹B³
A²A²/B¹B³
Isozyme composition
B¹B¹
B¹B²
A¹B¹
B²B²
Electrophoretic phenotype
A¹B²
A²B¹
B¹B³
A²B²
A¹A¹
B²B³
A¹A²
A¹B³
A²A²
A²B³
B³B³


Phenotype designation
A¹A¹/B²B²
A¹A²/B²B²
A²A²/B²B²
A¹A¹/B²B³
A¹A²/B²B³
A²A²/B²B³
A¹A¹/B³B³
A¹A²/B³B³
A²A²/B³B³
Isozyme composition
B²B²
Electrophoretic phenotype
A¹B²
B²B³
A¹A¹
A²B²
A¹A²
A¹B³
B³B³
A²B³
A²A²

the existence of two alleles at the a locus, a^1 and a^2; and three alleles at the b locus, b^1, b^2 and b^3, thus predicting a total of 18 possible G-3-PDH phenotypes in lake whitefish white muscle. The assignment of numerals to alleles corresponds to the electrophoretic mobility of their respective subunit products. Thus, isozymes of composition A^2A^2 migrate faster than A^1A^1, and B^3B^3 migrates faster than B^2B^2 which migrates faster than B^1B^1. Isozymes containing two different subunits are intermediate in electrophoretic mobility to that of the two homodimers of the two different subunits. The isozymes A^2A^2, A^2B^3 and B^3B^3 (Figure 7a) are apparently similar in electrophoretic mobility and efforts to separate them using citric acid-amine buffers in the pH range 6-9 as described by Clayton and Tretiak (1972) were unsuccessful.

Figure 7a shows, in six A^2A^2 homozygotes, the progressive mobility of isozymes containing the three B subunits. The more complex phenotypes containing both A^1 and A^2 subunits are shown in Figure 7b and c. It is apparent that the A^2A^2 isozyme stains less intensely than any of the BB isozymes and the A^1A^1 isozyme is very weakly stained (Figures 7 a,b,c). All phenotypes were readily distinguishable except for $A^1A^1B^3B^3$ and $A^1A^2B^3B^3$ where the weak staining of both A^1A^1 and A^1A^2 isozymes sometimes made it difficult to classify individual fish. Since the staining conditions used in this study do not necessarily reflect physiological conditions, it is probably unwise to attribute physiological

significance to differences in staining intensity of the various isozymes.

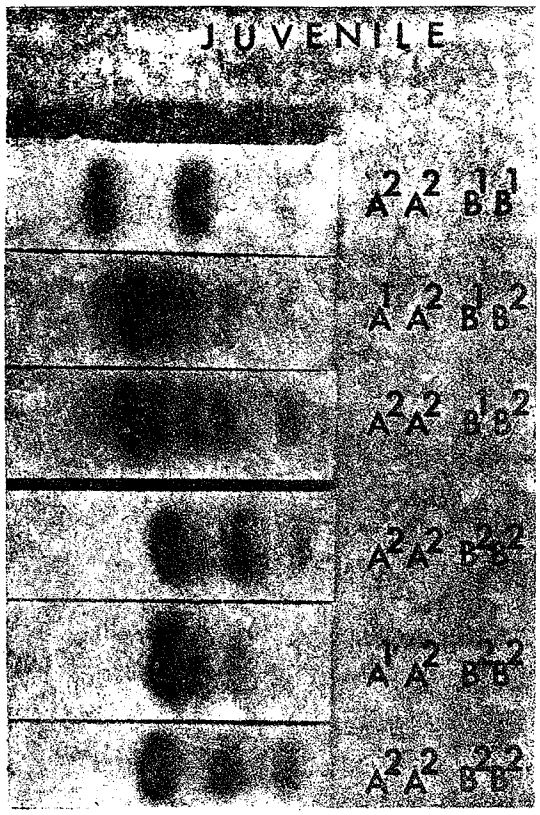
All eighteen phenotypes predicted by the model are illustrated in Figure 8 along with the subunit composition of isozyme bands. All of these phenotypes have been observed except $A^1A^1B^1B^1$, $A^1A^1B^1B^3$ and $A^1A^2B^1B^1$.

The G-3-PDH phenotypes found in laboratory reared juvenile lake whitefish are shown in Figures 9 a,b. The weak staining of isozymes that contain A^1 subunits is even more pronounced in juvenile fish than adults. Thus $A^1A^2B^3B^3$ juveniles (Figure 9 b) display only two isozymes in comparison to the expected three isozymes that were definitely visible in adult fish (Figure 7c). While this did not cause any difficulty in the progeny analysis, the weak staining of A^1 containing isozymes in the $A^1A^2B^1B^3$ and $A^1A^2B^2B^3$ phenotypes was more troublesome and it was impossible to classify a few of the juvenile fish.

Progeny from a total of 26 individual matings were analyzed in order to determine the heritability of the various phenotypes (Tables 2 and 3). Most of the parental fish were $\underline{a}^2\underline{a}^2$ homozygotes and results from all matings between $\underline{a}^2\underline{a}^2$ homozygotes are recorded in Table 2. The matings are arranged in Table 2 in order of increasing complexity, proceeding from matings between $\underline{bb}$ homozygotes to matings between unlike heterozygotes. Only two $\underline{a}^1\underline{a}^2$ heterozygotes were included in the breeding experiments and the results of three matings with these fish are shown in Table 3.

Figure 9(a,b) Electropherograms of G-3-PDH isozymes in muscle of laboratory reared juvenile lake whitefish.
Electrophoretic conditions outlined in Materials and Methods.

a


JUVENILE
$A^2A^2\ B^1B^1$
$A^1A^2\ B^1B^2$
$A^2A^2\ B^1B^2$
$A^2A^2\ B^2B^2$
$A^1A^2\ B^2B^2$
$A^2A^2\ B^2B^2$

b

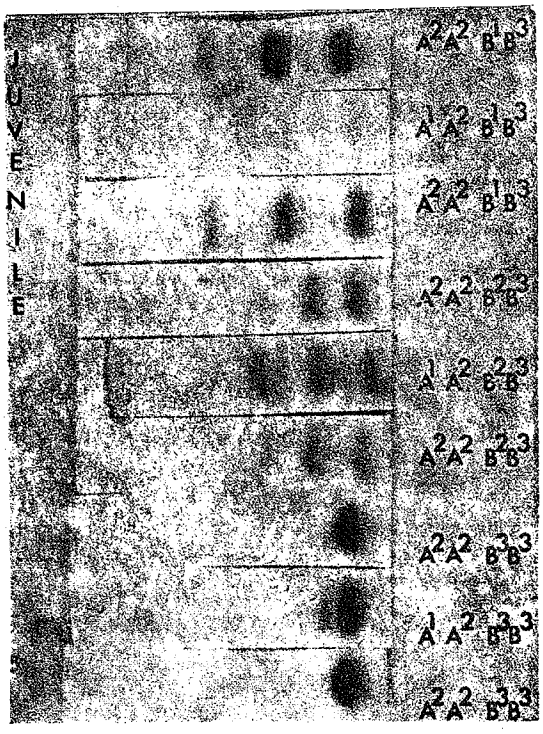

JUVENILE
$A^2A^2\ B^1B^3$
$A^1A^2\ B^1B^3$
$A^2A^2\ B^1B^3$
$A^2A^2\ B^2B^3$
$A^1A^2\ B^2B^3$
$A^2A^2\ B^2B^3$
$A^2A^2\ B^3B^3$
$A^1A^2\ B^3B^3$
$A^2A^2\ B^3B^3$

Table 2. Inheritance of BB phenotypes of glycerol-3-phosphate dehydrogenase in A^2A^2 homozygous lake whitefish.

Mating Identification[a] No.	Female x Male	Parental Phenotype Female x Male	Numbers of Progeny and Phenotypes B^1B^1	B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3	χ^2	p[b]
1	2060 x 2059	B^1B^1 x B^1B^1	32					1[c]	0.	1.0
2	2076 x 2078	B^3B^3 x B^3B^3						24	0.	1.0
3	3303 x 3308	B^1B^1 x B^3B^3				22			0.	1.0
4	2057 x 2058	B^3B^3 x B^1B^1				24			0.	1.0
5	3314 x 3311	B^2B^2 x B^1B^1		48						
6	3314 x 3319	B^2B^2 x B^1B^1		48						
	Total	B^2B^2 x B^1B^1		96					0.	1.0
7	3303 x 3309	B^1B^1 x B^1B^2	29	19					2.08	.25>p>.10
8	3312 x 3311	B^1B^2 x B^1B^1	26	22					0.33	.50>p>.25
9	3313 x 3311	B^1B^3 x B^1B^1	21			27				
10	3313 x 3319	B^1B^3 x B^1B^1	21			26				
	Total	B^1B^3 x B^1B^1	42			53			1.27	.50>p>.25
11	3322 x 3327	B^1B^2 x B^2B^2		21	27				0.75	.50>p>.25
12	3314 x 3320	B^2B^2 x B^1B^3		54			41		1.78	.25>p>.10
13	3324 x 3327	B^1B^3 x B^2B^2		21			27		0.75	.50>p>.25
14	2057 x 2056	B^3B^3 x B^1B^3	1[c]			25		20	0.56	.50>p>.25
15	2082 x 2084	B^1B^2 x B^3B^3				7	7		0.	1.0
16	3302 x 3307	B^2B^3 x B^3B^3					25	23	0.083	.90>p>.75
17	3312 x 3315	B^1B^2 x B^1B^2	10	26	12				0.57	.90>p>.25

...2/

Table 2 (Cont'd)

Mating Identification No.	Female x Male		Parental Phenotype Female x Male	Numbers of Progeny and Phenotypes B^1B^1	B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3	χ^2	p[b]
18	2062 x 2061		B^1B^3 x B^1B^3	1			12		8		
19	3313 x 3320		B^1B^3 x B^1B^3	25			40		31		
		Total	B^1B^3 x B^1B^3	26			52		39	4.33	.25>p>.10
20	3312 x 3317		B^1B^2 x B^2B^3		9	13	12	13			
21	3322 x 3326		B^1B^2 x B^2B^3		27	17	24	27			
22	3323 x 3326		B^1B^2 x B^2B^3		15	29	18	34			
		Total	B^1B^2 x B^2B^3		51	59	54	74		5.26	.25>p>.10
23	3313 x 3318		B^1B^3 x B^1B^2	19	11		20	14		3.37	.50>p>.25
24	3324 x 3326		B^1B^3 x B^2B^3		26		28	25	17	2.92	.50>p>.25

(a) Matings are identified by numbers 1-27 and by arbitrary numbers assigned to each parental fish.

(b) Probability of worse fit to simple Mendelian expectation of ratios of numbers of progeny.

(c) These fish were likely strays and were not included in α^2 calculation.

Table 3. Inheritance of glycerol-3-phosphate dehydrogenase phenotypes in lake whitefish.

Mating Identification[b]		Parental Phenotype		Numbers of Progeny and Phenotypes[a]							
No.	Female x Male	Female x Male		B^1B^2	B^2B^2	B^1B^3	B^2B^3	B^3B^3	χ^2	p[c]
25	3301 x 3304	$\frac{A^1A^2}{B^3B^3} \times \frac{A^2A^2}{B^2B^3}$	A^1A^2				10	12		
			A^2A^2	(3)[d]			8	15	2.38	.50>p>.25
26	3301 x 3305	$\frac{A^1A^2}{B^3B^3} \quad \frac{A^2A^2}{B^2B^3}$	A^1A^2				9	14		
			A^2A^2				11	14	1.50	.75>p>.50
27	3302 x 3306	$\frac{A^2A^2}{B^2B^3} \quad \frac{A^1A^2}{B^1B^2}$	A^1A^2	14	9	14	15			
			A^2A^2	16	29	22	18		15.0	.05>p>.03

(a) Progeny phenotypes are entered in columns for BB and rows for AA; thus 10 A^1A^2/B^2B^3 progeny were observed from mating 25. Progeny with A^1A^1 and B^1B^1 phenotypes were neither expected nor observed and these classifications are omitted from the table.

(b) Matings are identified by number 1-27 and by arbitrary numbers assigned to each parental fish.

(c) Probability of worse fit to simple Mendelian expectation of ratios of numbers of progeny.

(d) These fish were likely strays and are not included in d^2 calculations.

These breeding data (Tables 2 and 3) appear to provide convincing support for the genetic and molecular model for lake whitefish white muscle G-3-PDH isozymes. There is no example of the absence of any expected phenotypes among the progeny. On the other hand, while almost 1500 progeny were analyzed, only 5 (matings 1, 14, 25) were of unexpected phenotypes. These fish were also analyzed for malate dehydrogenase phenotype and while the genetics of this isozyme system are yet to be elucidated, the phenotypes were also irregular with respect to this enzyme. It is therefore suggested that these fish were most likely strays from other matings. The agreement with the model was evaluated quantitatively by comparing the observed numbers of progeny to the expected ratios via the standard χ^2 test (Tables 2 and 3). In all matings except 18, 19, and 27 the probability of worse agreement to the model exceeded 0.1.

In mating 27 it was necessary to distinguish between A^1A^2 and A^2A^2 phenotypes and as mentioned earlier some of these distinctions were difficult because of the faint staining of isozymes that contain the A^1 subunits. This is likely the reason that it was not possible to classify 7 of the 144 progeny from this mating that were analyzed. It is possible that inclusion of these samples could have made a slight improvement in the probability of the results. Samples from many lake whitefish populations in Western Canada were assayed for G-3-PDH phenotype distribution and in all but one case observed frequencies of alleles present in the various populations agree substantially with predictions based on the

Hardy-Weinberg principle (Table 10 A, B). In those populations where size classes of all phenotypes observed were large enough to permit analysis, the fit to expected numbers was within prescribed limits (Table 10 A, B).

The occurrence of multiple isozymes of G-3-PDH has been reported previously in several species of animals including some fishes. Johnson et al. (1970) reported three phenotypes of G-3-PDH in a Pacific rockfish, Sebastodes alutus but only one gene with two alleles apparently is involved. Similarly, McCabe et al. (1970) reported the existence of a single locus with three alleles for G-3-PDH in skipjack tuna, Katsuwonus pelamis but in their case the electropherograms are complicated by "artifact bands" which were not explained. Engel et al. (1971), however, in their studies of the salmonid tetraploidy hypothesis, have shown the existence of three genes coding for G-3-PDH subunits in a herring (Clupea harengus), a smelt (Osmerus eperlanus) and two species of trout (Salmo irideus (=S. gairdneri) and S. trutta). These authors concluded that the existence of the same number of genes coding for G-3-PDH in both reputedly diploid and tetraploid fishes neither supported nor refuted the tetraploidy hypothesis. Lake whitefish follow a system similar to that shown by Engel et al. (1971) with three G-3-PDH genes active in red muscle. In agreement with these authors, Clayton et al. (1973) named the most anodally migrating isozyme CC. However in lake whitefish, no isozymes of type AC are formed, unlike those clearly demonstrated in the species studied by Engel et al. (1971), thus perhaps little emphasis should

be placed on possible isozyme homologies between distantly related species. Clayton et al. (1973) also showed three phenotypes of G-3-PDH in one population of the cisco, Coregonus artedii, a species which is closely related to the lake whitefish. This species apparently has a set of three b alleles similar to lake whitefish but only one allele at the a locus. An interesting point here is that in the cisco, the supposed $A^2A^2B^3B^3$ phenotype forms the expected three isozyme bands, unlike the lake whitefish in which all three bands are of coincident mobility.

One possible way of testing isozyme homologies between species, is by comparing relative rates of heat inactivation of the various types of subunit. Clayton et al. (1973) found that on a relative basis, lake whitefish A^1A^1 and A^2A^2 isozymes are quickly inactivated by heat compared to little effect of heat on isozymes containing only B subunits. On the other hand, these authors found no differential activity between the two types of isozymes when they were tested with nicotinamide adenine dinucleotide and the coenzyme analogues nicotinamide-hypoxanthine dinucleotide and acetylpyridine-adenine dinucleotide. It was shown by Kaplan and Ciotti (1961) that these measures were effective in distinguishing between MDH isozymes.

Malate Dehydrogenase (MDH)

Malate dehydrogenase is an important enzyme of the energy producing citric acid cycle. Specifically, MDH catalyzes the reaction; malic acid + $NAD^+ \rightleftharpoons$ oxaloacetic acid + NADH + H^+ (White et al., 1964). MDH is a dimeric enzyme in higher organisms (Murphy

et al., Kitto, MS 1966) and in all species studied exists in two forms; mitochondrial MDH, found mainly in mitochondria; and supernatant MDH, found mainly in cell cytoplasm (Thorne et al., 1963). Evidence from many species of vertebrates has shown that the two forms of MDH are usually quite different in physiological properties, electrophoretic mobility and genetic control (Kaplan and Ciotti, 1961; Bailey et al., 1969; Bailey et al. 1970; Clayton et al., 1971). Also it is apparent that the similarity of supernatant MDH's between species is greater than the similarity of the two types of MDH within a single species (Kitto, MS 1966).

In the last few years MDH has been studied in a variety of fish species including sunfishes (Centrarchidae) (Whitt et al., 1973), the Pacific saury (Scomberesocidae) (Numachi, 1970), the walleye and sauger (Percidae) (Clayton and coworkers, 1970, 1973) and salmon and trout (Salmonidae) (Bailey et al., 1969; Bailey et al., 1970). Most pertinent here, Bailey et al. (1969) and Bailey et al. (1970) devised a model based on chemical, immunological and genetic considerations to explain their observations of electrophoretic phenotypes of king salmon (Oncorhynchus tshawytscha) and rainbow trout supernatant MDH. Initially the model proposed that there were two genes for supernatant MDH yielding two types of subunits, A and B, thus producing the commonly observed king salmon phenotype having three isozyme bands composed of AA, AB and BB dimers. The discovery of some six-banded phenotypes led Bailey et al. (1969) to propose that there were two alleles, b and b' at the b locus, leading to the increased

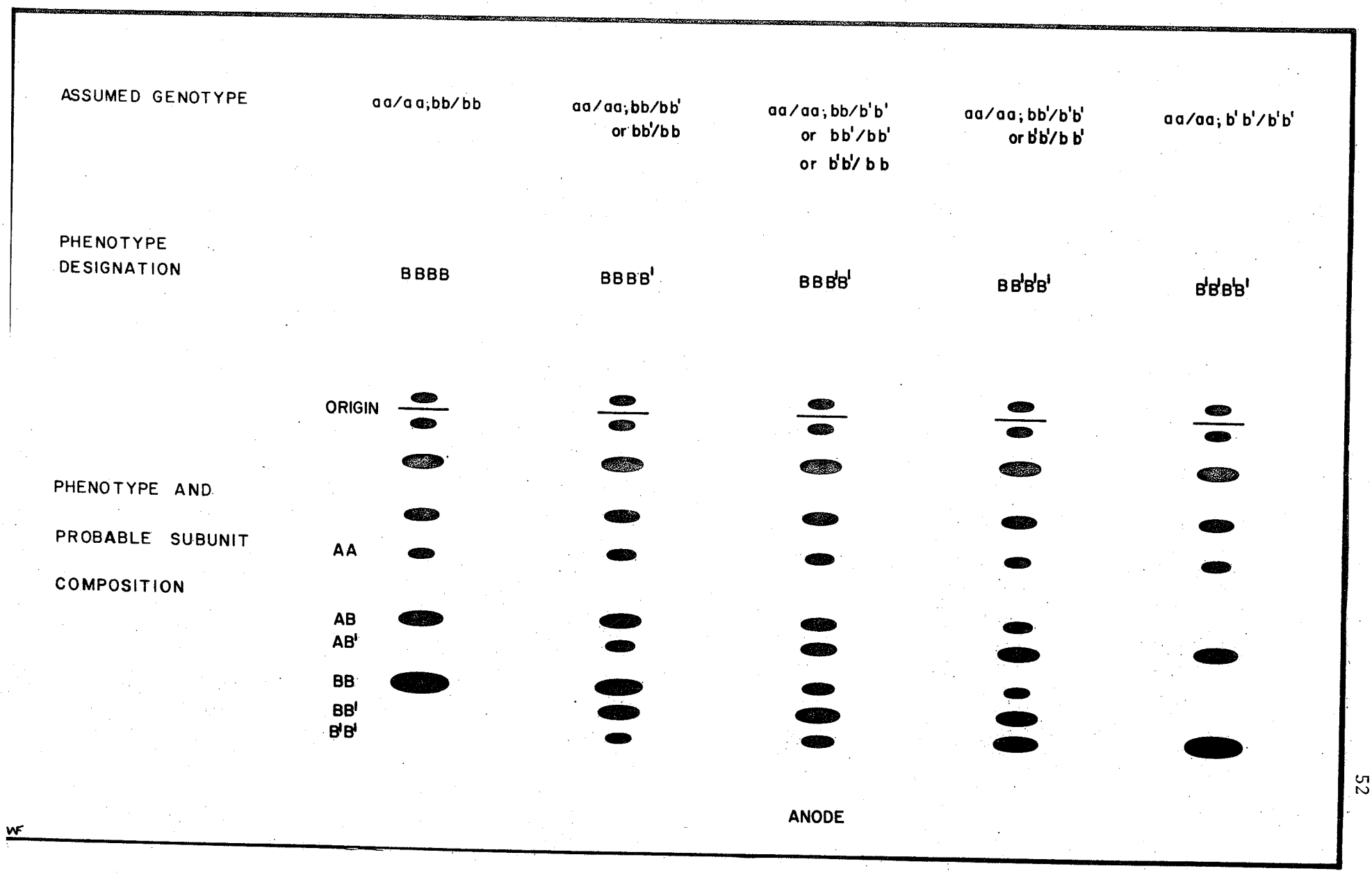
number of dimers. However, densitometric studies of relative staining intensities of isozymes coupled with the results of a breeding experiment on king salmon forced Bailey et al. (1970) to further revise the model to one in which the b gene not only possessed two alleles, but was also duplicated. This feature of the model satisfactorily explained asymmetric ratios in the activity of the BB, BB', B'B' set of isozymes when it was allowed that either b allele could be present at from 0 to 4 doses on the pair of b genes. Bailey et al. (1970) did their breeding experiment with king salmon only; the results from this experiment realistically may confirm only the action of two alleles at one of the b loci. These authors intuitively decided that the same two gene system applies to rainbow trout and used evidence from the trout to support the model. Rainbow trout examined by Bailey et al. (1970) show a similar variation to that of king salmon. The difference between the two is in the frequency of the alleles b and b' in their two populations. In their king salmon population the b allele is in high frequency and only bb/bb and bb/bb' individuals were recognized. In their rainbow trout population the b' allele is in high frequency and b'b'/b'b' and b'b'/b'b individuals made up the bulk of the population with a few bb/b'b' individuals recorded. In neither species did Bailey et al. (1970) recognize all five types of supernatant MDH phenotypes as predicted by their model. Numachi et al. (1972) provided additional support for the Bailey et al. model through their studies of rainbow trout and chum salmon. These authors found an additional expected phenotype (BBBB') as proposed by the model in their rainbow trout populations.

Numachi et al. also show evidence of a b^2 allele in chum salmon, if one accepts that the genes in different salmonid species are in fact identical.

Lake whitefish provide better evidence for the Bailey et al. (1970) model than any species studied to date. In central Canadian lake whitefish populations all five of the supernatant MDH phenotypes predicted by the Bailey et al. (1970) model are observed (Figure 10). Although the same general type of designation for MDH isozymes as used by Bailey et al. (1970) has been used in this study it is not implied that the genes for MDH in the lake whitefish are identical to those of king salmon and/or rainbow trout. The use of a similar nomenclatural system is purely of convenience for comparative purposes.

Data from the lake whitefish breeding experiment (Table 4) generally fit the model when parental fish are classified by red muscle phenotype, but difficulties arise with some parental fish that have different MDH phenotypes for red and white muscle. Nine parental fish involved in six matings fall into this category. In each case the red muscle phenotypes indicate an increase in staining intensity of subunits coded by b allele (but not b^1) perhaps indicating greater activity of the b allele in red compared with white muscle in these individuals. Since progeny were killed at an early age, at a size when it is impossible to separate the two muscle types, results of matings involving parents with "dual" muscle MDH phenotypes remain confused. It is not clear what phenotypes to expect in progeny from matings in which one or both parental fish had different MDH phenotypes for the two muscle types.

Figure 10 Diagram of lake whitefish supernatant malate dehydrogenase phenotypes showing assumed subunit composition of the isozymes. Electrophoretic conditions outlined in Materials and Methods.


ASSUMED GENOTYPE
aa/aa;bb/bb
aa/aa;bb/bb'
or bb'/bb
aa/aa;bb/b'b'
or bb'/bb'
or b'b'/bb
aa/aa;bb'/b'b'
or b'b'/bb'
aa/aa;b'b'/b'b'
PHENOTYPE
DESIGNATION
BBBB
BBBB'
BBB'B'
BB'B'B'
B'B'B'B'
ORIGIN
PHENOTYPE AND
PROBABLE SUBUNIT
COMPOSITION
AA
AB
AB'
BB
BB'
B'B'
ANODE

WF

Table 4. Inheritance of supernatant malate dehydrogenase phenotypes in muscle of lake whitefish.

Mating No.[a]	Parental Phenotypes		Numbers of Progeny and Phenotypes						
	Female	Male	BBBB	$BBBB^1$	BBB^1B^1	$BB^1B^1B^1$	$B^1B^1B^1B^1$	χ^{2d}	p[e]
25	BBBB	$B^1B^1B^1B^1$			45			0.	1.0
26	BBBB	$BB^1B^1B^1$		20	28			1.34	.1<p<.25
13	$BB^1B^1B^1$	BBBB		23	26			0.188	.5<p<.75
16[b]	$BB^1B^1B^1$	BBBB		15	21	7			
20	BBB^1B^1	BBBB		48				0.	1.0
7	$BBBB^1$	BBBB	22	26				0.334	.5<p<.75
11	$BBBB^1$	BBBB	25	19				0.818	.25<p<.5
23	BBB^1B^1	$B^1B^1B^1B^1$			2	63		0.062	.75<p<.9
12	$BBBB^1$	$BBBB^1$	24	42	22			0.273	.75<p<.9
6	$BBBB^1$	BBB^1B^1		27	20			1.04	.25<p<.5
19[c]	BBB^1B^1	$BBBB^1$		38	52	3			
5[c]	$BBBB^1$	BBB^1B^1		9	26	10			
21[c]	$BBBB^1$	BBB^1B^1	1	14	43	10			
22[c]	$BBBB^1$	BBB^1B^1	4	24	56	12			
3	$BBBB^1$	$BB^1B^1B^1$		5	14	3		2.01	.25<p<.5
27	$BB^1B^1B^1$	$BBBB^1$		18	52	20		2.37	.25<p<.5
24	$BB^1B^1B^1$	BBB^1B^1			20	27		1.04	.5<p<.75
8	BBB^1B^1	BBB^1B^1		2	46				
17[c]	BBB^1B^1	BBB^1B^1		6	39	1			
9[c]	BBB^1B^1	BBB^1B^1		1	27	16			
10	BBB^1B^1	BBB^1B^1		3	45				

...cont'd

Table 4 (cont'd)

[a] Corresponds to mating identification numbers in G-3-PDH experiment, Tables 2 and 3.

[b] Progeny analysis poor.

[c] Matings in which at least one parent had a "dual" muscle MDH phenotype (see Text).

[d] Calculated χ^2 value.

[e] Probability of worse fit to simple Mendelian expectations of ratios of numbers of progeny.

This problem may have its source in the regulation of the activity of MDH genes. Clayton et al. (1970), Clayton et al. (1973) found that in walleye and sauger liver synthesis at the most anodal supernatant MDH locus (not duplicated in these fishes) apparently is shut off entirely and only the a gene is active. Bailey et al. (1969) reported that the activity of the a gene(s) predominates in liver of salmon and trout. This latter condition is true in lake whitefish also (Clayton, Pers. Comm.). This type of partial regulation of MDH genes seen in liver may occur for the b genes in muscle tissues of lake whitefish individuals in which red and white muscle yield different MDH phenotypes. Just how this regulation takes place cannot be determined through the simple genetic experiments undertaken in the present study. Supernatant MDH has been implicated in at least three metabolic roles which operate to different extents in different tissues. Since AA and BB isozymes exhibit some tissue specificity in at least some fishes (e.g. walleye and sauger, salmon and trout-reference above) it is possible that in salmonid fishes (including lake whitefish) gene duplication has allowed functional differentiation of different types of BB isozymes (Bailey et al., 1970).

Bailey et al. (1970) were unable to distinguish between the symmetrical heterozygous genotypes bb'/bb' and bb/b'b' in king salmon because of the restricted nature of their breeding experiment. This has been possible for four matings in the lake whitefish experiment. Coincidentally all of these matings involve the bb/b^1b^1 type, the one producing only one type of gamete bb^1; the other matings involving the two symmetrical types of heterozygotes were those in which difficulties arose because of "dual" phenotypes

in white and red muscle. No linkage of the two b genes is indicated by any of the matings which fit expectations based on the Bailey et al. (1970) model. Bailey et al. (1970) found this to be the case for king salmon as well.

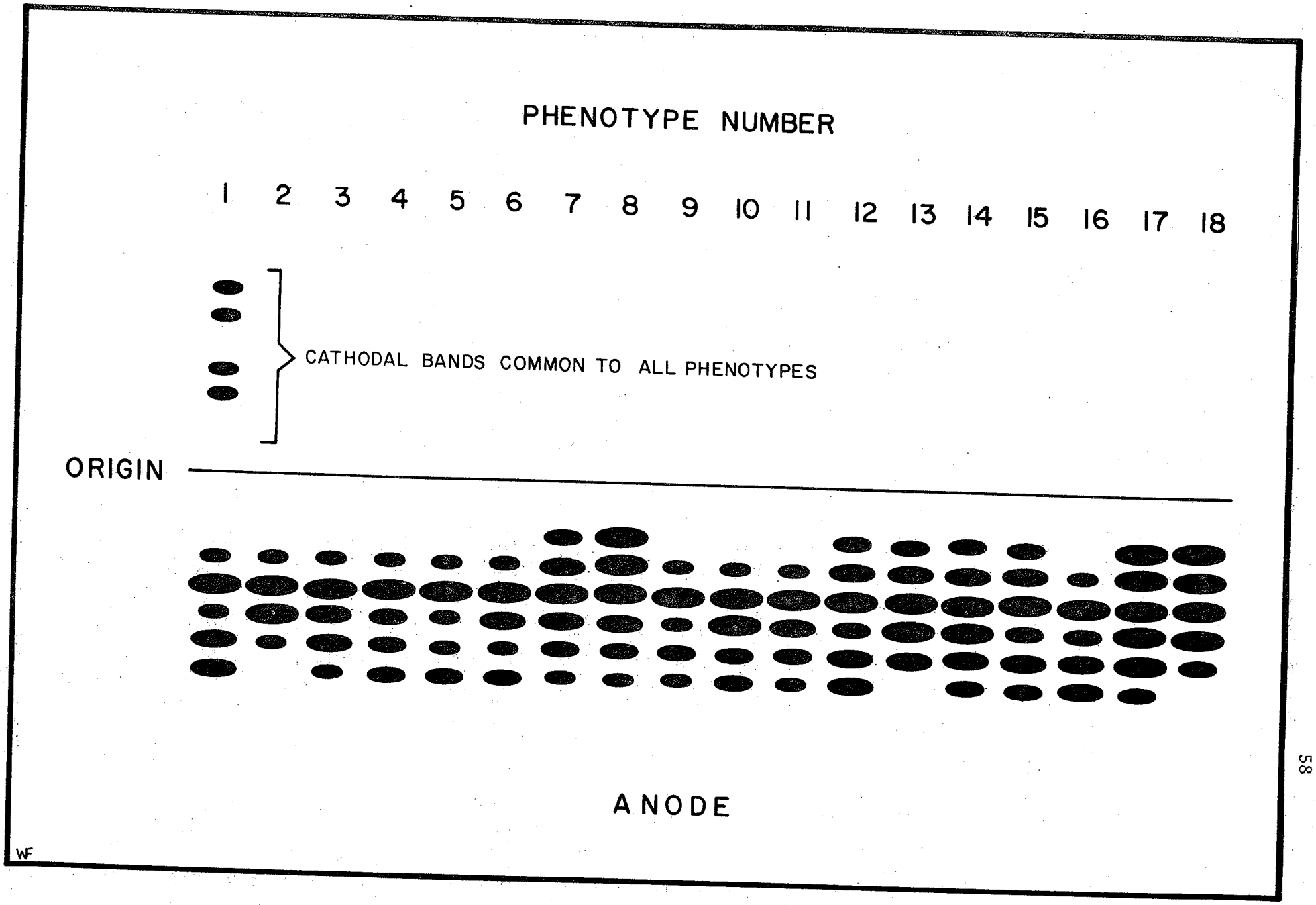
It was unfortunate that the "dual" phenotype situation was not discovered before all progeny had been sacrificed. Another breeding experiment, in which progeny would be reared to a size from which red muscle could be sampled, may be required before the genetics of lake whitefish muscle supernatant MDH can be completed.

Hemoglobin

The existence of polymorphic hemoglobin phenotypes visualized via electrophoresis has been demonstrated for many species of fishes. Salmonid fishes, as a group, possess probably the widest variation in this regard; some species of Prosopium appear to be monomorphic over vast distances (unpublished data) while lake whitefish exhibit 18-20 different hemoglobin electrophoretic phenotypes within a similar range. Usually not all of these phenotypes (Figure 11) are found in a single lake whitefish population. However, in a large sample from a large multi-basin lake in central Canada (e.g. Lake Athapapuskow, Manitoba) most of the phenotypes may be observed.

Lack of knowledge about the molecular structure of lake whitefish hemoglobin was one of the difficulties which prevented completion of a genetic explanation of observed electrophoretic phenotypes. Generally vertebrate hemoglobins are composed of two

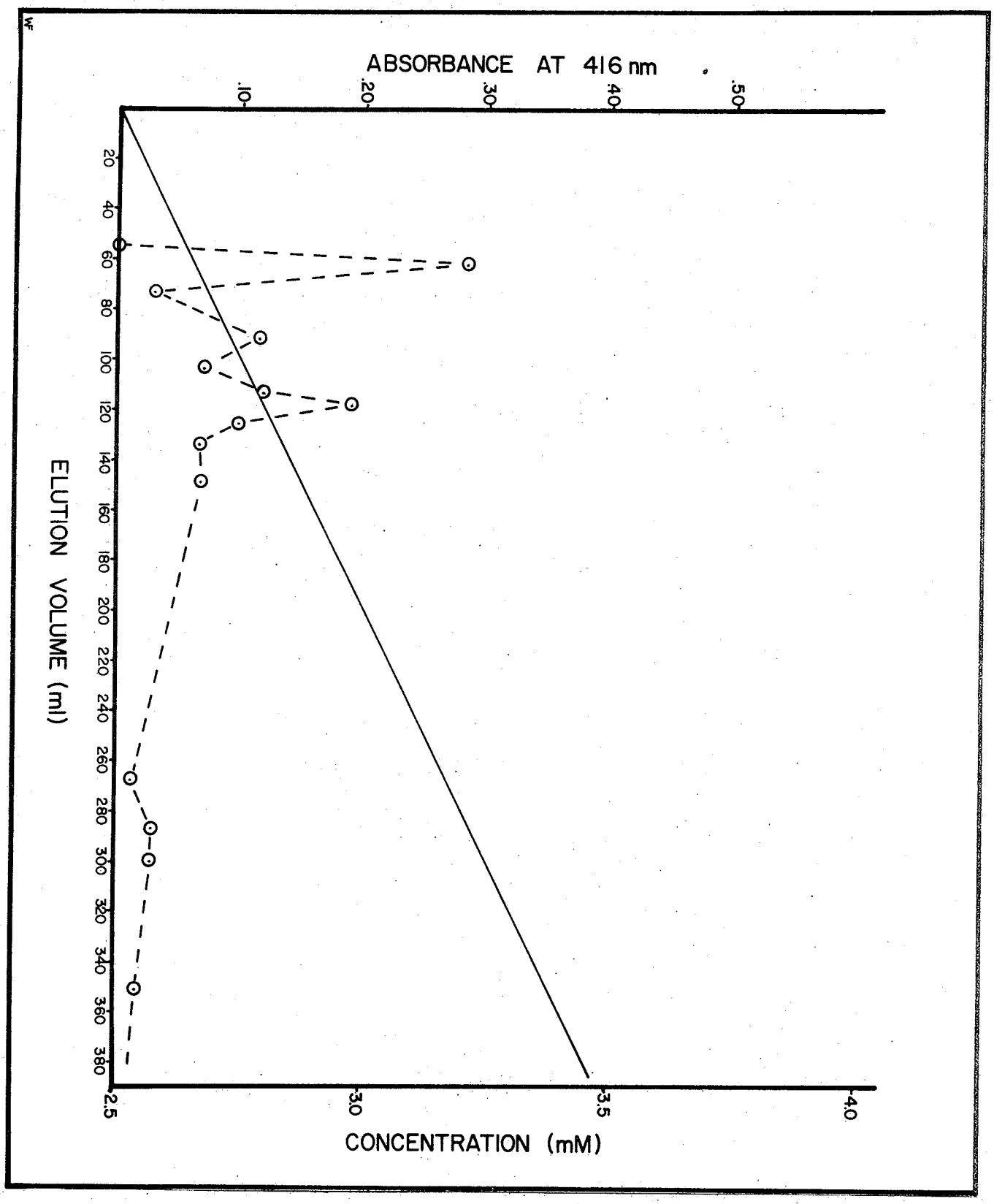
Figure 11. Diagram of observed hemoglobin phenotypes in lake whitefish. Electrophoretic conditions outlined in Materials and Methods.


PHENOTYPE NUMBER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
CATHODAL BANDS COMMON TO ALL PHENOTYPES
ORIGIN
ANODE
WF

each of two types of globin polypeptides (e.g. α and β chains of human hemoglobin A) thus yielding the common hemoglobin tetrameric structure (αβαβ). However, recent work on both humans (Efremov et al., 1969) and some fish species (Wilkins, 1968; Powers and Edmundson, 1972) has shown that some animals may produce asymmetric hemoglobin tetramers of the type AABC or even ABCD. It is not known whether or not lake whitefish produce these types of hemoglobin tetramers. Also, it is not known how many genes code for hemoglobin polypeptides in lake whitefish. Commonly up to three or four genes may be active in mammals; if lake whitefish do carry the double diploid number of genes as is suspected, as many as eight genes may code for hemoglobin subunits.

Considerable effort was directed toward solution of these two problems. A necessary first step was the large scale separation of the various bands seen in lake whitefish hemoglobin electropherograms in order to provide sufficient material for further study. Ion exchange chromatography using DEAE Sephadex A25 and A50 and Whatman DE 32 cellulose over several conditions of pH, ionic strength, buffer combinations, gradients, temperature, flow rates and phosphate additives proved fruitless. These techniques were similar to those found to be successful with hemoglobins of other fish species (Powers and Edmundson, 1972; Gillen and Riggs, 1972; Li, Tomita and Riggs, 1972). An example of one of the best elution profiles obtained is shown in Figure 12. Two specific difficulties were encountered in elution of lake whitefish hemoglobins on ion exchange columns. First, the elution buffer system had to contain

Figure 12 Illustration of the elution profile of hemoglobin phenotype number 8 eluted from a Whatman DE 32 ion exchange column. Two chamber gradient in concentration; 0.0025 M and 0.005 M $Na_4P_2O_7$ adjusted to pH 8.5 with concentrated H_3PO_4. Sample diluted 1:5 in the 0.0025 M buffer prior to introduction to column. Flow rate approximately 19.5 ml /hr.


ABSORBANCE AT 416 nm
.10
.20
.30
.40
.50
ELUTION VOLUME (ml)
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
2.5
3.0
3.5
4.0
CONCENTRATION (mM)

a phosphate component at low ionic strength to prevent precipitation of the hemoglobin sample on contact with the ion exchanger. Secondly, in experiments with buffers that contained phospate the hemoglobin sample would enter the column and subdivide into bands similar to that seen on electropherograms but, before eluting from the column, the bands would once again coalesce into large broad peaks. This phenomenon apparently was due to strong attraction of the hemoglobin to the column. The ionic strength of the buffer which would entirely elute fast-moving components apparently was sufficient to elute the slower components as well.

Several of the systems used separated the electrophoretically anodal and cathodal groups of bands very well, but consistent within-group separations were not obtained. Whether the hemoglobin used was in carbon monoxy-, cyanmet- or oxidized form had no effect, either in ion exchange columns or in electrophoretic gels.

Urea starch gel electrophoresis, after the methods of Smithies et al. (1972) and Tsuyuki (Pers. Comm. to Dr. J.W. Clayton) proved to be unsuccessful at determining the number of globin chains in lake whitefish hemolysates or ion exchange column fractions. Hemolysates appeared to be altered beyond separation of the globin chains by the conditions of these methods (pH 2-3) and difficulties were encountered with sample size of the ion exchange columm fractions, when fractionation was achieved.

Experiments with starch gel electrophoresis, in which a series in ionic strength of orthophosphate, pyrophosphate and inositol

hexaphosphate (IHP) were added to hemolysates, gel and box buffers (pH 8.5 boric acid-NaOH) showed that at concentrations of the order of 0.005M of these additives the whole hemoglobin electrophoretic phenotypes were shifted anodally, in relation to the number of phosphate groups present. That is, IHP moved the bands farther than pyrophosphate which moved bands farther than orthophosphate. Associated with the increased anodal movement of the phenotypes was an increased proximity of bands one to another. Evidently the phosphate groups bound onto the hemoglobin molecules, thus resulting in their increased movement electrophoretically by virtue of increased negative charge. This feature of lake whitefish hemoglobin probably is linked to the difficulties with elution of hemolysates on ion exchange columns in which phosphates were not present in the eluting buffer. Apparently elution of lake whitefish hemolysates on ion exchange columns with buffers containing no phosphate component removes a "native" phosphate entity important in the physiological properties and stereochemical conformation of lake whitefish hemoglobin. Maintenance of a given level of some phosphate component in the elution buffers prevents the "stripping" of the "native phosphate" and denaturation does not occur. Studies on human hemoglobin (Benesch and Benesch, 1969) have shown that 2, 3 diphosphoglycerate (2,3 DPG) is closely linked to hemoglobin molecules and is much involved in the oxygenation-deoxygenation reaction. However it has not been shown that 2, 3 DPG or another phosphate is necessary in elution buffers for ion exchange chromatography. Li, Tomita and Riggs (1972) found that 2,

3 DPG had no effect on oxygen equilibria of hagfish hemoglobins, nor did ATP, which is also known to effect oxygen equilibria of hemoglobins of higher vertebrates. Gillen and Riggs (1972), however, have shown that ATP does affect the physiological reactions of carp hemoglobins and this phosphate may be the "2, 3 DPG" of fishes generally. As in the human hemoglobin studies, neither Li et al. nor Gillen and Riggs reported any problems with denaturation of hemoglobins on ion exchange columns in the absence of phosphate although their various methods were slightly different than those of the present study. At present the native phosphate group in lake whitefish blood is unknown but it may very well be ATP.

The above vacuities in the knowledge of lake whitefish hemoglobin molecular structure has prevented thorough genetic analysis of data from the breeding experiment. However, a little can be gained from the breeding experiment.

Part of the electrophoretic phenotypes - the least anodally migrating portion of the anodal group of bands - allows the separation of phenotypes into three subclasses (Figure 13, also see Figure 11). Data from the breeding experiments (Table 5) coupled with data from field collections (Table 6) suggests that these three subclasses are the result of the action of two non-dominant alleles at a single locus. For convenience these suggested alleles were designated L and 1. Despite the convincing nature of the breeding data, a satisfactory genetic molecular model to explain these subclasses was not devised. The lack of basic structural data,

as outlined previously, together with the poor fit of some populations to Hardy-Weinberg expectations (based on a two allele system) precludes definite conclusions on the inheritance of electrophoretic phenotypes of whitefish hemoglobin.

II Population Studies

Results of Field Collections

Lake whitefish samples of fourteen or more individuals were obtained from the locations indicated in Figure 14. Results of electrophoretic analyses of extracts of red and white muscle for products of LDH heart type alleles and alleles of G-3-PDH *a* and *b* genes are shown in Table 7 along with geographic position, drainage basin, elevation and mean July air temperature for each lake.

Two important assumptions are implicit in the interpretation of these data: 1) that identical electrophoretic phenotypes of these protein characters from individual fish taken from different locations are the products of identical genotypes; and 2) that lake whitefish from all the locations sampled are really members of a single species, in the sense of Mayr's biological species. There are risks in making these assumptions. Without determining amino acid sequences of the subunits of each of the characters it is not certain that they are identical, even though they may have identical electrophoretic mobility. Salthe (1969) demonstrated immunological methods which enabled him to differentiate some identical electrophoretic phenotypes in animals from widely-separated geographic locations. Also, without in some way testing the concept of a single species over the wide distribution of lake whitefish it is not certain that

Figure 13 Diagram of three "subclasses" of lake whitefish hemoglobin phenotypes.

SUGGESTED ALLELIC CONFIGURATION

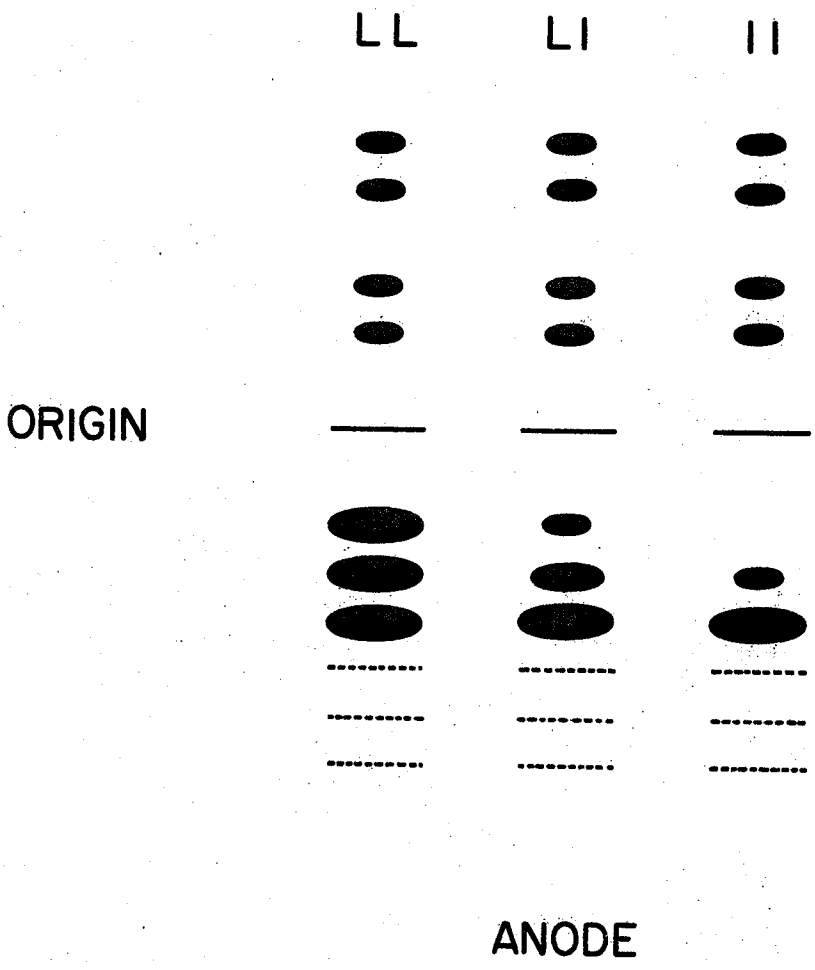


Table 5. Inheritance of two postulated alleles controlling the cathodal part of the anodal group of bands in lake whitefish hemoglobin phenotypes.

Matings	Progeny Phenotypes		
	LL	L1	11
LL x LL			
2082 x 2084 (1968)[a]	17	0	0
LL x L1			
3302 x 3307	45	24	0
3302 x 3306	47	51	0
Total	92	75	0
LL x 11			
3303 x 3308	0	25	0
3318 x 3313	0	42	0
2059 x 2060 (1968)	0	15	0
Total	0	82	0
L1 x 11			
3317 x 3312	0	22	26
3301 x 3305	0	22	26
3301 x 3304	0	30	41
3327 x 3322	0	26	42
2057 x 2056 (1968)	0	22	25
2078 x 2076 (1968)	0	5	5
Total	0	127	165
L1 x L1			
3324 x 3327	8	29	15
11 x 11			
3303 x 3309	0	0	59
3312 x 3311	0	0	48
3313 x 3319	0	0	72
3313 x 3320	0	0	72
3314 x 3320	0	1	46
3314 x 3319	0	0	74
3314 x 3311	0	0	14
2057 x 2058 (1968)[b]	0	6	32
2076 x 2077 (1968)[b]	1	20	11
Total	1	27	428

[a] From 1968 breeding experiment

[b] Progeny analyses poor.

Table 6. "Zygotic frequencies" and "gene frequencies" of a postulated one gene--two allele system controlling the cathodal part of the anodal group of bands in lake whitefish hemoglobin phenotypes. Hardy-Weinberg expectations in brackets.

Lake	LL	L1	11	Frequency of L "Allele"
Yukon System				
Aishihik	25 (25.27)	6 (5.4)	0 (0.29)	0.902
Laberge	24 (23.19)	3 (3.65)	0 (0.01)	0.927
Squanga	58 (58.)	0 (0.)	0 (0.)	1.000
Liard System				
Cache	23 (23.)	2 (1.92)	0 (0.)	0.960
Watson	2 (4.1)	10 (5.8)	0 (2.1)	0.584
Fraser System				
Lahache	0 (0.)	1 (0.98)	34 (34.02)	0.014
Peace System				
Moberly	0 (0.14)	4 (3.75)	24 (24.11)	0.072
Athabasca System				
Talbot	0 (0.)	0 (0.)	10 (10.)	0.000
Mackenzie System				
Great Slave	1 (1.6)	11 (9.8)	14 (14.6)	0.250
Churchill System				
Churchill R. Mouth	12 (13.1)	23 (20.7)	7 (8.1)	0.560
S. Saskatchewan System				
Waterton	0 (0.)	0 (0.)	19 (19.)	0.000

...2/

Table 6. (cont'd)

Lake	LL	Ll	11	Frequency of L "Allele"
N. Saskatchewan System				
Big Athapap	12 (8.2)	61 (68.6)	147 (143.2)	0.193
Little Athapap	1 (2.02)	9 (6.97)	5 (5.86)	0.367
Clear	27 (27.2)	41 (40.6)	15 (15.2)	0.572
Clearwater	0 (0.04)	2 (1.9)	26 (26.0)	0.021
Steeprock	6 (6.4)	4 (3.2)	0 (0.4)	0.800
North Winnipeg	6 (7.64)	28 (24.60)	18 (19.71)	0.384
Wabamun	11 (15.6)	26 (16.9)	0 (4.6)	0.648

Figure 14 Collection sites for lake whitefish populations indicated in Table 7. Numbers on map correspond to those of Table 7.

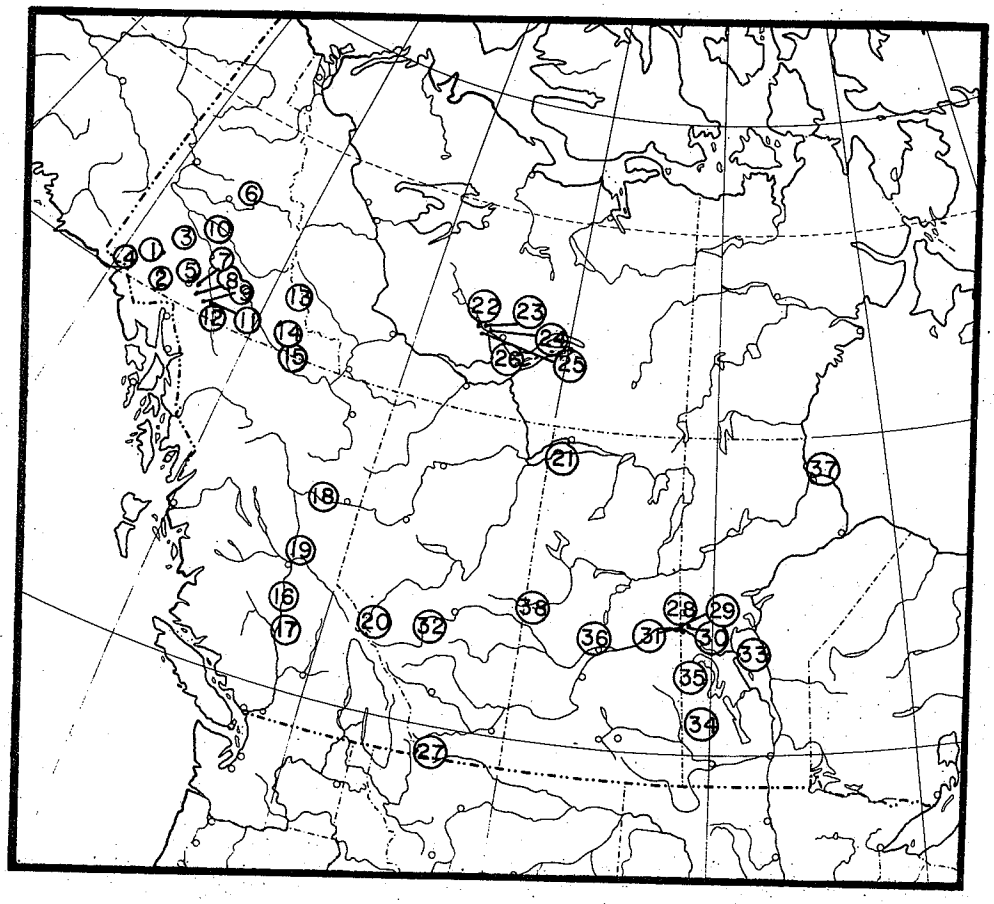

6
10
3
1
7
5
2
8
9
13
12
11
14
15
22
23
24
26
25
21
37
18
19
16
17
20
32
38
28
29
36
31
30
33
35
34
27

Table 7. Gene frequencies (glycerol-3-phosphate a^1, b^1, b^2 and b^3 alleles and LDH h^b allele), sample size and geographic and climatic data for 38 collections of lake whitefish. N_1=N for G-3-PDH analysis; N_2=N for LDH analysis.

	Lake	Lat.°	Long.°	Elev.	MJT[a]	$F^b(a^1)$	$F(b^1)$	$F(b^2)$	$F(b^3)$	$F(h^b)$	N_1	N_2
	YUKON											
	Alsek System											
1.	Aishihik L.	61.50	137.25	3001	55	0.000	0.000	0.815	0.185	0.000	62	-[c]
2.	Dezadeash L.	60.50	137.00	2305	55	0.040	0.000	0.444	0.556	0.000	63	63
	Yukon System											
3.	Fox L.	61.25	135.50	2580	57	0.000	0.000	0.648	0.360	0.000	43	-
4.	Kluane L.	61.17	138.50	2563	55	0.000	0.000	0.357	0.643	0.000	14	-
5.	Laberge L.	61.00	135.08	2060	57	0.148	0.000	0.704	0.296	0.000	27	30
6.	Mayo L.	63.78	135.33	2200	59	0.166	0.000	0.722	0.278	0.000	18	-
7.	McLintock L.	60.58	133.92	2500	57	0.050	0.000	0.733	0.267	0.000	30	-
8.	Snafu L.	60.18	133.43	2500	57	0.000	0.000	0.795	0.205	0.000	22	-
9.	Squanga L.	60.47	133.63	2590	57	0.223	0.000	0.382	0.618	0.000	251	58
10.	Tatchun L.	62.28	136.12	1500	57	0.000	0.000	0.481	0.519	0.000	26	-
11.	Teenah L.	60.30	133.42	2500	57	0.232	0.000	0.432	0.568	0.000	158	-
12.	Little Teslin L.	60.48	133.48	2500	57	0.139	0.000	0.278	0.722	0.000	18	-
	Liard System											
13.	Cache L.	61.95	128.23	4250	57	0.000	0.000	1.000	0.000	0.814	21	24
14.	Frances L.	61.25	129.30	2540	59	0.011	0.000	0.831	0.169	0.000	59	29
15.	Watson L.	60.10	128.80	2230	59	0.000	0.000	0.978	0.022	0.000	23	27
	NON YUKON											
	Fraser System											
16.	Lac la Hache	51.82	121.50	2650	61	0.000	0.000	0.300	0.700	0.674	80	83
17.	Williams L.	52.12	122.08	1859	61	0.000	0.000	0.591	0.409	0.136	22	22
	Peace System											
18.	Moberly L.	53.82	121.75	2270	61	0.007	0.000	0.535	0.465	0.908	71	71
19.	Summit L.	54.28	122.67	2315	59	0.000	0.000	0.792	0.208	0.990	48	48

...2/

Table 7. (cont'd)

	Lake	Lat.°	Long.°	Elev.	MJT[a]	F[b](a^1)	F(b^1)	F(b^2)	F(b^3)	F(h^b)	N_1	N_2
	Athabasca System											
20.	Talbot L.	53.08	118.00	3287	57	0.000	0.000	0.740	0.260	0.886	48	48
21.	Athabasca L.	59.42	110.00	699	65	0.024	0.397	0.183	0.420	0.825	63	63
	Mackenzie System											
22.	Alexie L.	62.68	114.07	725	59	0.011	0.444	0.211	0.345	0.986	45	37
23.	Baptiste L.	62.72	114.23	686	59	0.000	0.180	0.330	0.490	0.972	50	53
24.	Chitty L.	62.72	114.12	729	59	0.012	0.329	0.354	0.317	0.987	41	38
25.	Drygeese L.	62.75	114.17	712	59	0.019	0.154	0.019	0.827	0.875	107	104
26.	Great Slave L.	62.00	114.00	518	59	0.000	0.364	0.272	0.364	0.896	22	24
	South Saskatchewan River											
27.	Waterton L.	49.05	113.90	4193	57	0.000	0.625	0.025	0.350	0.800	20	20
	North Saskatchewan System											
28.	Big Athapap L.	54.57	101.42	956	61	0.006	0.281	0.303	0.416	0.950	160	50
29.	Little Athapap L.	54.62	101.67	956	61	0.013	0.397	0.359	0.244	0.977	39	43
30.	Clearwater L.	54.00	101.55	855	65	0.000	0.411	0.191	0.398	0.950	143	30
31.	Narrows	54.60	101.45	956	61	0.023	0.386	0.136	0.478	0.983	22	29
32.	Wabamun L.	53.53	114.58	2380	63	0.037	0.046	0.630	0.324	0.882	54	55
33.	North Winnipeg L.	53.00	98.00	713	65	0.018	0.225	0.311	0.464	0.949	111	108
34.	Clear L.	50.67	100.00	2017	65	0.000	0.381	0.131	0.488	0.714	80	77
35.	Steeprock L.	52.58	101.42	2300	65	0.019	0.250	0.317	0.433	0.135	52	52
	Churchill System											
36.	Crean L.	54.08	106.17	1720	61	0.000	0.091	0.114	0.795	0.955	22	22
37.	Churchill R. Mouth	58.83	94.17	000	54	0.014	0.429	0.243	0.328	0.957	35	46
38.	Primrose L.	54.92	109.75	1964	61	0.000	0.133	0.400	0.467	0.933	30	30
	Totals										2200	1348

[a]MJT = Mean July Temperature
[b]F( )= Frequency of allele in brackets
[c]Yukon populations were considered homozygous for LDH h^c allele after an unbroken run of 151 fish from three areas because the probability of encountering the other allele by further sampling had dropped to less than 5%.

this assumption holds. Lindsey et al. (1970) referred to Coregonus clupeaformis as a "species complex"--certainly any species with restricted movement and a distribution as vast as that of lake whitefish is likely to have geographical races but the extent of divergence of any of these supposed races is unknown. There are circumpolar species, e.g. Esox lucius, which are commonly considered to be single species; yet undoubtedly these types of species show morphological variation and have gaps in their distributions. Presumably North American lake whitefish are similar.

In the context of geological history, there are two possible explanations for the observed distributions of lake whitefish population gene frequencies across Western Canada. First, one could ascribe the observed distributions to a postglacial history of mutation and selection continuing to the present. Under this hypothesis, bearing in mind the considerable geographic range of the lake whitefish, one would expect to observe the result of selection as clines in gene frequencies in response to climatic or other environmental gradients.

Alternatively, the observed genetic variation could be the result of postglacial admixture of two or more slightly different genetic stocks of lake whitefish, subsequent to a period of mutation and possibly differential selection in the two or more widely separated glacial refugia. Under this hypothesis, one would expect a more haphazard array of population gene frequencies with a major influence exerted by routes of postglacial dispersal.

Several general considerations suggest that the latter hypothesis is correct. These are: 1) Mutation and selection require time to show

their effects. The Wisconsin glacial period, throughout which lake whitefish were isolated into two main refugia, lasted some 40,000 years or more compared with about 5,000-10,000 years of post-glacial time. 2) During the glaciation, the climate, day length, growing season etc. in the two major refugia must have differed considerably, thus favoring the development of differences between the two lake whitefish groups. 3) The events of deglaciation, as outlined in the introduction, were conducive to the wide dispersal of fishes, making the hypothesis of admixture of lake whitefish stocks probable. 4) Lake whitefish exhibit behavioral preference for cool conditions, e.g. remaining below the thermocline in lakes in which surface waters become very warm, thus minimizing the effects of changing temperatures on their habitats. 5) The lake whitefish distribution is discontinuous, yet over a wide geographic area of central Canada, an area with a postglacial history of watershed interconnections, population gene frequencies are similar. This situation may be explained in two ways--either the present-day selection pressures over the area are rather uniform and the observed gene frequencies reflect those of ancestral migrant populations, or the whole area has been colonized and recolonized, the degree of gene flow between groups being sufficient to maintain a large relatively homogeneous group of populations. This latter case may be more plausible -- there are water connections between many of the lakes in present times, and it does not require that selection operate in a uniform fashion over a large area.

Support for a postglacial selection hypothesis would be provided by correlation between population gene frequencies and climatic or

environmental gradients. Tables 8 A, B and C show simple correlation coefficients for the variables in Table 7; A) when all the data are taken together, B) when Yukon River samples are treated alone and C) when non-Yukon River samples are treated alone. This breakdown of the data was made on the basis of an apparent geological barrier to lake whitefish movement and hence gene flow into Yukon watersheds from without, and because G-3-PDH $\underline{b}^1$ and LDH $\underline{h}^b$ alleles are totally absent from Yukon collections. This break is also supported by the distribution of hemoglobin and MDH phenotypes which will be discussed later. The independent indications of a break in gene flow on the part of each of the biochemical characters was considered justification for the division of populations in the simple correlation calculations. It is immediately evident that most of the correlation found when all samples are taken together is removed by the separation of the samples into the Yukon and non-Yukon groups. A logical conclusion is that much of the correlation found initially was the result of a mixture of two at least partially discrete groups of populations.

In the Yukon group, only negative correlation between G-3-PDH $\underline{a}^1$ frequency and longitude remained (Table 8 (B)). A close examination of the area (Figure 15) reveals that the direction of the decline in population frequencies of G-3-PDH $\underline{a}^1$ is coincident with the downstream direction of the major rivers of the area, Yukon and Teslin rivers. The highest frequencies of G-3-PDH $\underline{a}^1$ are seen in Squanga and Teenah lakes; if the allele had its origins in headwaters such as these, the present day distribution of the allele in this area is very similar to what would be expected on the basis of spread by lake

Table 8(A). Simple correlation coefficients: lake whitefish field collections, all samples. Significance at 5%*=0.320, at 1%**=0.413, N=38, df=36.

	Variable	1	2	3	4	5	6	7	8
Latitude	1	1.000							
Longitude	2	0.563**	1.000						
Elevation	3	-0.106	0.527**	1.000					
Mean July T	4	-0.559**	-0.639**	0.092	1.000				
G-3-PDH a^1	5	0.307	0.382*	0.124	-0.220	1.000			
G-3-PDH b^1	6	0.300	-0.789**	-0.477**	0.393*	-0.295	1.000		
G-3-PDH b^2	7	0.298	0.618**	0.335*	-0.337*	0.072	-0.723**	1.000	
LDH h^b	8	-0.431**	-0.780**	-0.356*	0.461**	-0.464**	0.638**	-0.487**	1.000

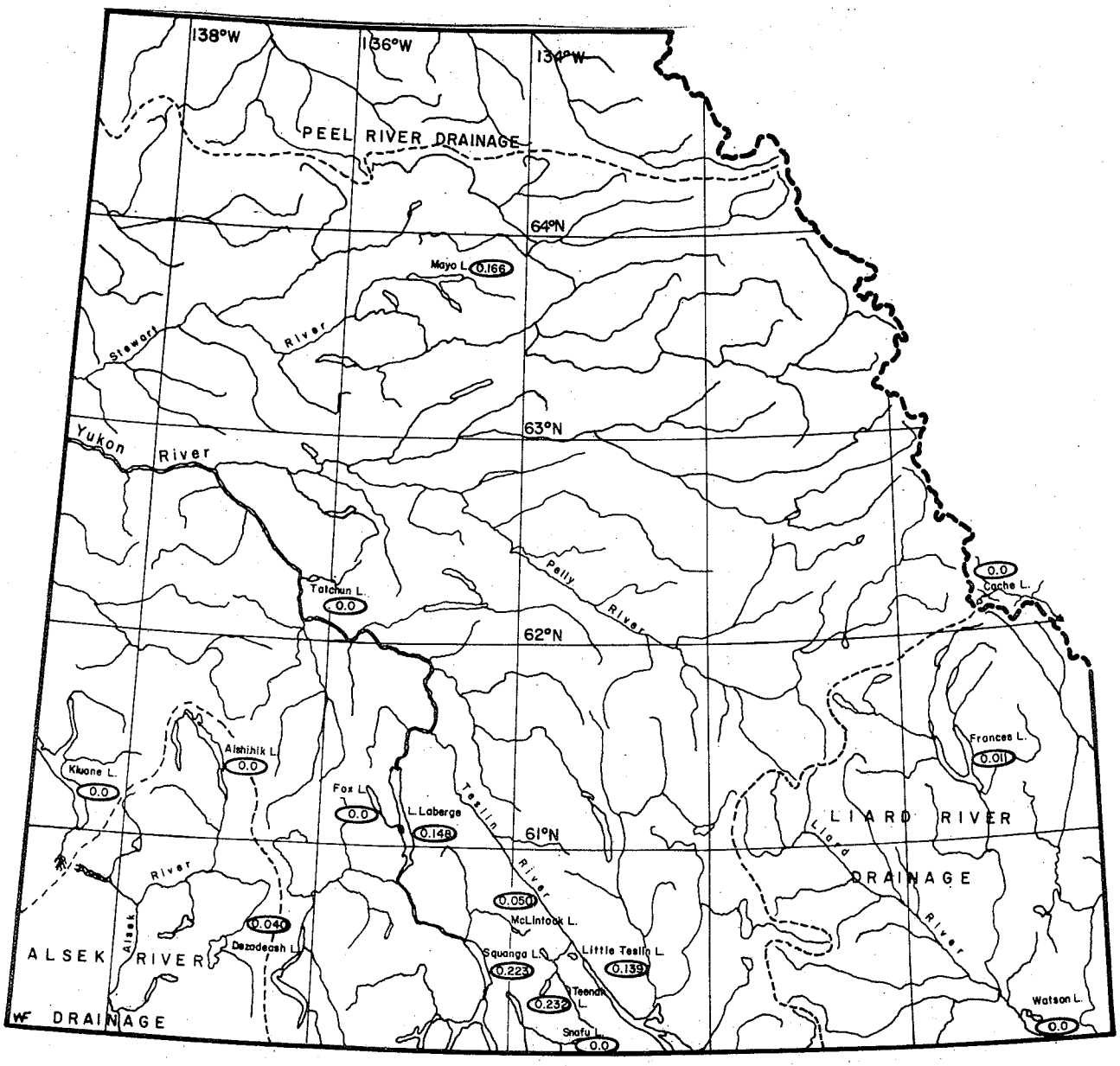
Table 8 (B) Simple correlation coefficients: lake whitefish field collections, Yukon River samples (including Alsek River). Significance at 5%*=0.576, at 1%**=0.708. N=12, df=10.

Variable		1	2	3	4	5	6	7	8
Latitude	1	1.000							
Longitude	2	0.378	1.000						
Elevation	3	-0.415	-0.060	1.000					
Mean July T	4	0.423	-0.643*	-0.332	1.000				
G-3-PDH a^1	5	-0.055	-0.580*	0.042	0.504	1.000			
G-3-PDH b^1	6	0.000	0.000	0.000	0.000	0.000	0.000		
G-3-PDH b^2	7	0.279	-0.004	0.099	0.202	-0.320	0.000	1.000	
LDH h^b	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 8 (C) Simple correlation coefficients: lake whitefish field collections, non-Yukon River samples. Significance at 5%*=0.388, at 1%**=0.496, N=26, df=24.

	Variable	1	2	3	4	5	6	7	8
Latitude	1	1.000							
Longitude	2	0.291	1.000						
Elevation	3	-0.300	0.527**	1.000					
Mean July T	4	-0.394*	-0.348	0.347	1.000				
G-3-PDH a[1]	5	0.100	-0.305	-0.218	0.296	1.000			
G-3-PDH b[1]	6	-0.010	-0.684**	-0.411*	0.093	0.188	1.000		
G-3-PDH b[2]	7	0.154	0.660**	0.293	-0.233	-0.164	-0.747**	1.000	
LDH h[b]	8	0.003	-0.409*	-0.251	-0.073	0.074	0.382	-0.440*	1.000

Figure 15 Distribution of gene frequencies of G-3-PDH $\underline{a}^1$ allele over a relatively small area in Yukon Territory.


138°W
136°W
134°W
PEEL RIVER DRAINAGE
64°N
Mayo L.
0.166
Stewart
River
63°N
Yukon River
Pelly River
Tatchun L.
0.0
0.0
Cache L.
62°N
Frances L.
0.011
Aishihik L.
0.0
Kluane L.
0.0
Fox L.
0.0
L. Laberge
0.148
Teslin River
61°N
LIARD RIVER
DRAINAGE
Liard River
Alsek River
0.040
Dezadeash L.
ALSEK RIVER
0.050
McLintock L.
Squanga L.
0.223
Little Teslin L.
0.139
Teenda L.
0.232
Snafu L.
0.0
Watson L.
0.0
DRAINAGE

whitefish dispersal. During deglaciation it is very likely that many of these Yukon lakes e.g. Squanga, Teenah, Little Teslin and McLintock were connected to each other and to the larger lakes to which they are now tributary (Laberge, Teslin) either by proglacial and glacial lakes or by meltwater streams (Mulligan, 1963). Unfortunately, data for some of the larger lakes in the area such as Teslin, Atlin, Tagish and Bennett, are lacking, preventing comparison of these lakes to the headwater lakes. That G-3-PDH $\underline{a}^1$ was not found in Aishihik, Fox, Kluane, Snafu, and Tatchun lakes may in some cases be due to accident of sampling and in others due to accident of colonization. Finally, the negative correlation between G-3-PDH $\underline{a}^1$ frequencies and longitude seen in these Yukon populations is readily explained on the basis of postglacial dispersal of lake whitefish without recourse to the actions of selection.

Following separation of samples into the two groups, six significant correlation coefficients remained among the variables for the non-Yukon group of samples. Two of these, negative correlation between frequencies of G-3-PDH $\underline{b}^2$ allele and frequencies of both G-3-PDH $\underline{b}^1$ allele and LDH $\underline{h}^b$ allele are probably merely the result of their opposing correlation with longitude with which frequencies of $\underline{b}^2$ allele are positively correlated. Similarly, the strong positive correlation between elevation and longitude probably accounts for the negative correlation of G-3-PDH $\underline{b}^1$ allele frequencies with elevation. Therefore only negative correlation of G-3-PDH $\underline{b}^1$ and LDH $\underline{h}^b$ and positive correlation of G-3-PDH $\underline{b}^2$, with longitude remain important. As mentioned in the introduction, postglacial events favored the northwestward dispersal of fishes from a Mississippi

refugium; thus one might expect a genetic character originating in this refugium to show negative correlation with longitude. The fact that G-3-PDH $\underline{b}^1$ allele is absent from Yukon watersheds, Fraser River watershed and the western headwaters of the northern plains rivers (North Saskatchewan, Athabasca and Peace rivers) suggests that the occurrence of this allele has been governed by the post-glacial dispersal of lake whitefish from a Mississippi refugium. A similar scheme fits the occurrence of the LDH $\underline{h}^b$ allele, although its more widespread occurrence (i.e. into Fraser River watersheds and northern plains river headwaters) suggests that perhaps lake whitefish colonization of western central Canada came in two phases, an early wave in which LDH h^b was carried and G-3-PDH $\underline{b}^1$ was not and a later wave in which this G-3-PDH allele was carried.

G-3-PDH $\underline{b}^2$ allele is ubiquitous[1] in all populations sampled but positive correlation was found between population frequencies of this allele and longitude. There are three possible explanations for this observation: 1) Lake whitefish in both refugia possessed the $\underline{b}^2$ allele, but the development of the $\underline{b}^1$ allele in the Mississippi refugium early in postglacial time led to a partial displacement of the $\underline{b}^2$ allele in early southern populations, through selective advantage of the $\underline{b}^1$ allele. 2) Lake whitefish in both refugia

---

[1] G-3-PDH $\underline{b}^3$ allele is also ubiquitous, but calculations using frequencies of this allele provide no further information once both $\underline{b}^2$ and $\underline{b}^1$ frequencies have been used to determine correlation coefficients. $\underline{b}^2$ frequencies were chosen over $\underline{b}^3$ because $\underline{b}^2$ frequencies were generally the higher of these two G-3-PDH alleles found in Yukon populations.

possessed the $\underline{b}^2$ allele, but the greater contribution of Bering refugium emigrants carrying only $\underline{b}^2$ and $\underline{b}^3$ G-3-PDH alleles raised the population frequencies of the $\underline{b}^2$ allele in western central Canadian lake whitefish populations. 3) the G-3-PDH $\underline{b}^2$ allele had its origin in the Bering refugium and its presence as far east as Hudson Bay is the result of postglacial dispersal. The last hypothesis is supported by the presence of LDH $\underline{h}^c$ allele equally far eastward and also G-3-PDH $\underline{a}^1$ allele both of a supposed Bering origin. That the Bering form of Prosopium cylindraceum, the round whitefish, has spread all the way to Hudson Bay (McPhail and Lindsey, 1970) adds potentiality to this hypothesis. G-3-PDH $\underline{b}^2$ allele is absent from some eastern Canadian lake whitefish populations that have been examined (unpublished data); this also supports a Bering refugium for the $\underline{b}^2$ allele although the far eastern lake whitefish populations may have been derived from an Atlantic refugium. The above points make the third hypothesis, a Bering origin for G-3-PDH $\underline{b}^2$ allele, the most likely although the other two, particularly the second, cannot be entirely ruled out.

From the preceding discussions it seems clear that the significant correlation coefficients obtained between population frequencies of LDH $\underline{h}^b$, G-3-PDH $\underline{a}^1$, $\underline{b}^1$ and $\underline{b}^2$ and longitude, and the overall distributions of gene frequencies of these characters are the result of postglacial dispersal of lake whitefish from their two glacial refugia into central Canada and B.C., rather than the result of a postglacial history of mutation and selection.

Tables 9 and 10 (A and B) show observed frequencies of LDH heart

Table 9. Observed phenotype frequencies and Hardy-Weinberg expectations for LDH in non-Yukon populations of lake whitefish. Expected frequencies in brackets.

Lake	SS	FS	FF	χ^2	P
Liard System					
Cache L.	17 (15.90)	5 (7.27)	2 (0.83)		
Frances L.	0 (0.0)	0 (0.0)	29 (29.0)		
Watson L.	0 (0.0)	0 (0.0)	27 (27.0)		
Fraser System					
Lac la Hache	45 (39.8)	25 (35.3)	13 (7.8)	7.2	0.050>P>0.025
Williams L.	2 (0.41)	2 (5.17)	18 (16.42)		
Peace System					
Moberly L.	59 (58.54)	11 (11.86)	1 (0.60)		
Summit L.	47 (47.04)	1 (0.95)	0 (0.00)		
Athabasca System					
Talbot L.	38 (37.68)	9 (9.70)	1 (0.62)		
Athabasca L.	43 (42.88)	18 (18.19)	2 (1.93)		
Mackenzie System					
Alexie L.	36 (36.97)	1 (1.02)	0 (0.01)		
Baptiste L.	50 (50.07)	3 (2.88)	0 (0.04)		
Chitty L.	37 (37.02)	1 (0.98)	0 (0.01)		
Drygeese L.	80 (79.63)	22 (22.75)	2 (1.63)		
Great Slave L.	19 (19.27)	5 (4.47)	0 (0.26)		
South Saskatchewan System					
Waterton L.	12 (12.80)	8 (6.40)	0 (0.80)		

...2/

Table 9 (cont'd)

Lake	SS	FS	FF	χ^2	P
North Saskatchewan System					
Big Athapap L.	45 (45.13)	5 (4.75)	0 (0.13)		
Little Athapap L.	41 (41.04)	2 (1.93)	0 (0.02)		
Clearwater L.	27 (27.08)	3 (2.85)	0 (0.08)		
Narrows	28 (28.02)	1 (0.97)	0 (0.01)		
Wabamun L.	44 (42.79)	9 (11.45)	2 (0.77)		
North Winnipeg L.	97 (97.26)	11 (10.45)	0 (0.28)		
Clear L.	39 (39.25)	32 (31.45)	6 (6.30)	0.026	0.900>P>0.750
Steeprock L.	2 (0.95)	10 (12.14)	40 (38.91)		
Churchill System					
Crean L.	20 (20.06)	2 (1.89)	0 (0.04)		
Churchill R. Mouth	42 (42.13)	4 (3.79)	0 (0.09)		
Primrose L.	26 (26.11)	4 (3.75)	0 (0.13)		

Table 10(A) Phenotype frequencies and Hardy-Weinberg expectations for G-3-PDH b alleles in Yukon populations of lake whitefish. Expected numbers in brackets.

Lake	B^2B^2	B^2B^3	B^3B^3	χ^2	P
Alsek System					
Aishihik L.	41 (41.2)	19 (18.7)	2 (2.1)		
Dezadeash L.	14 (12.4)	29 (31.1)	20 (19.5)	0.34	0.90>P>0.75
Yukon System					
Fox L.	22 (17.6)	11 (19.8)	10 (5.6)	8.54	0.03>P>0.01
Kluane L.	1 (1.8)	8 (6.4)	5 (5.8)		
Laberge L.	14 (13.4)	10 (11.3)	3 (2.4))		
Mayo L.	8 (9.4)	10 (7.2)	0 (1.4)		
McLintock L.	18 (16.1)	8 (11.7)	4 (2.1)		
Snafu L.	14 (13.9)	7 (7.2)	1 (0.9)		
Squanga L.	37 (36.6)	118 (118.5)	96 (95.9)	0.006	P>0.995
Tatchun L.	8 (6.0)	9 (13.0)	9 (7.0)	2.44	0.50>P>0.25
Teenah L.	27 (29.5)	80 (77.5)	51 (51.0)	0.288	0.90>P>0.75
Little Teslin L.	1 (1.4)	8 (7.2)	9 (9.4)		

Table 10 (B) Phenotype frequencies and Hardy-Weinberg expectations for G-3-PDH b alleles in non-Yukon populations of lake whitefish. Expected numbers in brackets.

Lake	B^1B^1	B^1B^2	B^1B^3	B^2B^2	B^2B^3	B^3B^3	χ^2	P
Liard System								
Cache L.	0(0.0)	0(0.0)	0 (0.0)	21(21.0)	0(0.0)	0(0.0)		
Frances L.				43(40.7)	12(16.6)	4(1.7)		
Watson L.				22(22.0)	1(1.0)	0(0.0)		
Fraser System								
Lac la Hache				5(7.2)	38(33.6)	37(39.2)	1.11	.95>P> .90
Williams L.				9(7.7)	8(10.6)	5(3.7)	1.4	0.75>P>0.50
Peace System								
Moberly L.				20(20.3)	36(35.3)	15(15.6)	0.03	0.99>P>0.98
Summit L.				30(30.1)	16(15.8)	2(2.1)		
Athabasca System								
Talbot L.				28(26.3)	15(18.5)	5(3.2)	1.7	0.50>P>0.25
Athabasca L.	8(9.9)	11(9.2)	23(21.0)	0(2.1)	12(9.7)	9(11.1)		
Mackenzie System								
Alexie L.	5(8.9)	14(8.4)	16(13.8)	1(2.0)	3(3.3)	6(5.4)		
Baptiste L.	2(1.6)	7(5.9)	7(8.8)	5(5.5)	16(16.2)	13(12.0)		
Chitty L.	3(4.4)	11(9.6)	10(8.6)	5(5.1)	8(9.2)	4(4.1)		
Drygeese L.	1(2.5)	0(0.6)	31(27.3)	1(0.0)	2(3.4)	72(73.2)		
Great Slave L.	4(2.9)	4(4.4)	4(5.8)	2(1.6)	4(4.4)	4(2.9)		
South Saskathewan System								
Waterton L.	7(7.8)	1(0.6)	10(8.8)	0(0.0)	0(0.4)	2(2.5)		

...2/

Table 10 (B) (cont'd)

Lake	B^1B^1	B^1B^2	B^1B^3	B^2B^2	B^2B^3	B^3B^3	χ^2	P
North Saskatchewan System								
Big Athapap L.	14(12.6)	31(27.3)	31(37.4)	14(14.7)	38(40.3)	32(27.7)	2.6	0.90>P>0.75
Little Athapap L.	9(6.2)	5(11.1)	8(7.6)	8(5.0)	7(6.8)	2(2.3)		
Clearwater L.	24(24.2)	16(22.5)	37(46.8)	5(5.2)	21(21.7)	20(22.7)	4.2	0.75>P>0.50
Narrows	5(3.3)	0(2.3)	7(8.1)	1(0.4)	4(2.9)	5(5.0)		
Wabamun L.	1(0.1)	2(3.1)	1(1.6)	23(21.4)	20(22.0)	7(5.7)		
North Winnipeg L.	7(5.6)	12(15.5)	24(23.2)	8(10.7)	41(32.0)	19(23.9)	5.4	.50>P> .25
Clear L.	10(11.6)	9(8.0)	32(29.8)	1(1.4)	10(10.2)	18(19.1))		
Steeprock L.	4(3.3)	8(8.2)	10(11.3)	6(5.2)	13(14.3)	11(9.8)	0.7	.99>P> .98
Churchill System								
Crean L.	0(0.2)	1(0.5)	3(3.2)	0(0.3)	4(4.0)	14(13.9)		
Churchill R. Mth.	7(6.4)	7(7.3)	9(9.9)	2(2.1)	6(5.6)	4(3.8)		
Primrose L.	1(0.5)	4(3.2)	2(3.7)	5(4.8)	10(11.2)	8(6.5)		

type and G-3-PDH B type phenotypes respectively. Calculated Hardy-Weinberg expectations are included in the tables, but in most cases numbers in the smallest expected classes are too few for tests of significance using χ^2. Among those cases where the χ^2 test was permitted, two populations showed significant deviation from Hardy-Weinberg expectations. In both cases--LDH phenotypes in Lac LaHache and G-3-PDH B phenotypes Fox Lake--heterozygote deficiency was detected. This observation will be discussed in a later section.

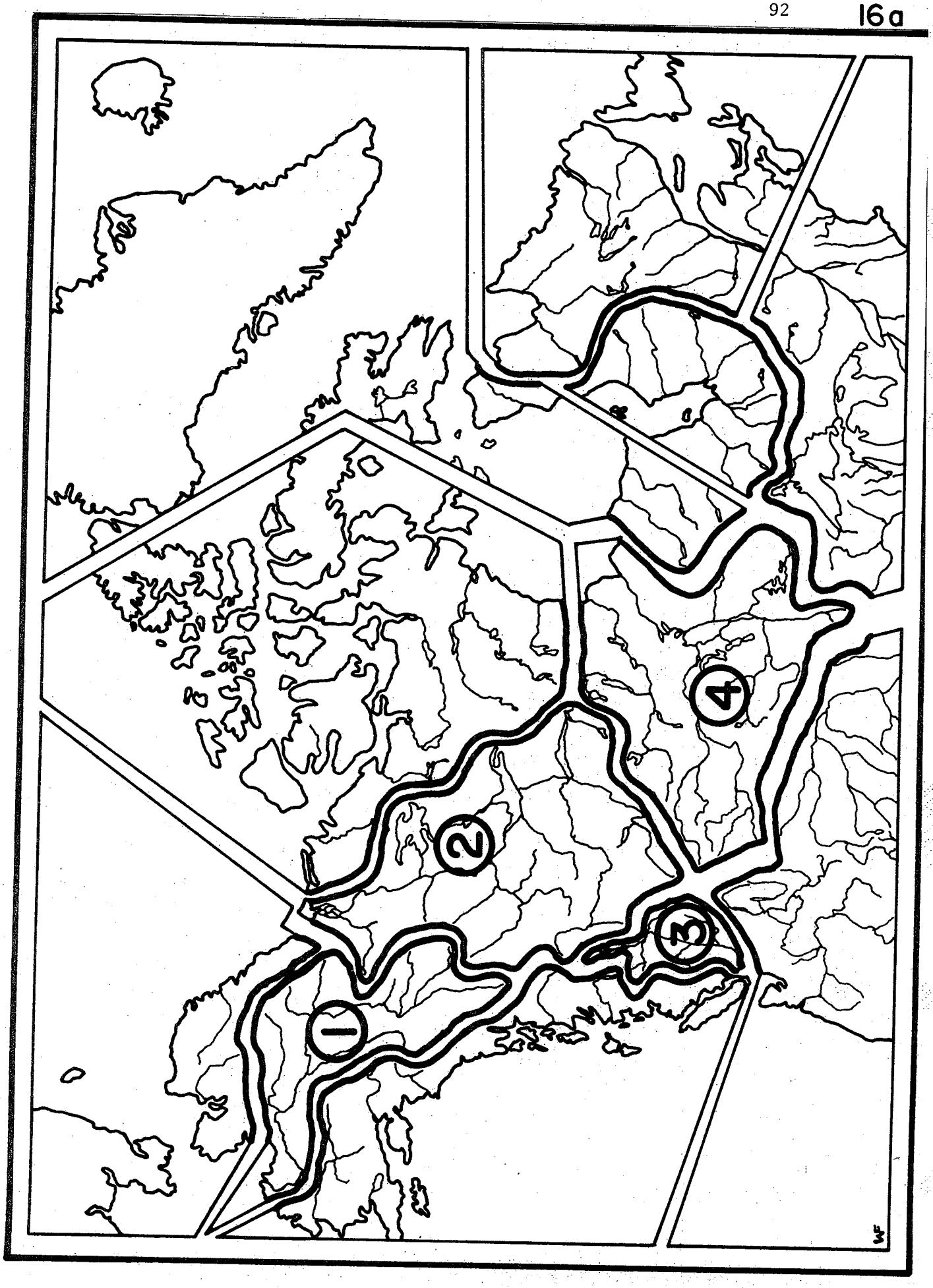
Biochemical characters, MDH and hemoglobin, for which the genetics of electrophoretic phenotypes are not fully understood provide limited information of zoogeographic significance .

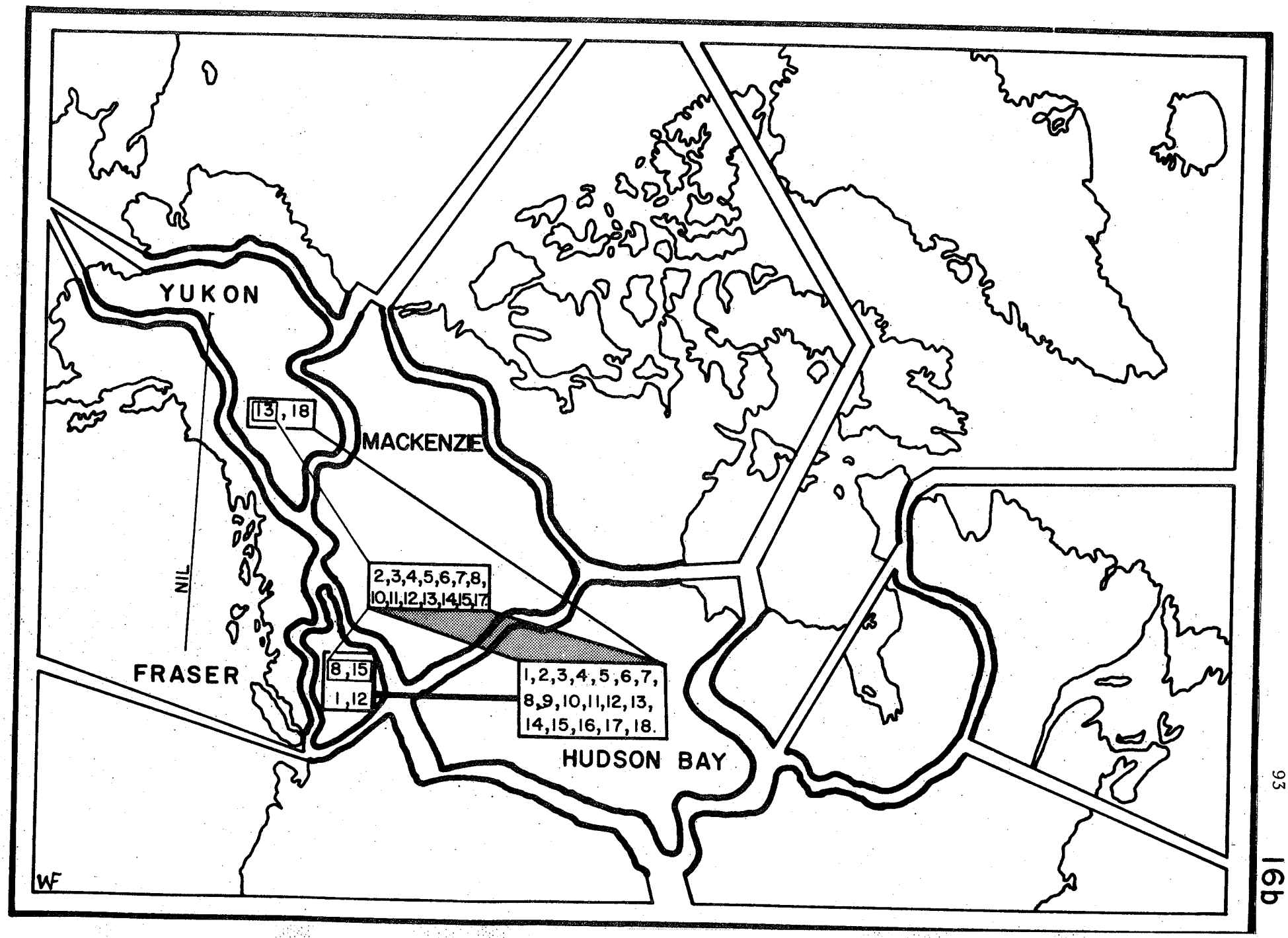
Hemoglobin pattern 1 (see Figure 11) is not found in Yukon populations at all while at the same time pattern 18 is found almost exclusively in Yukon populations. Frequencies of two postulated alleles controlling the least anodally migrating part of the anodal group of bands are shown in Table 6 (see section on hemoglobin genetic studies). Generally speaking, one of these "alleles" is in high frequency in Yukon populations and in quite low frequency in most other locations. The occurrence of the eighteen observed lake whitefish hemoglobin phenotypes (see Figure 11) provides further information on the affinities of lake whitefish populations. Figure 16(a) is a schematic representation of the major river basins in the study area. Figure 16(b) shows how the lake whitefish populations of these major watersheds are related based on the occurrence of the eighteen hemoglobin phenotypes. It

Figure 16 (a) Four major drainage basins of Central and Western Canada. 1) Yukon River, 2) Mackenzie River, 3) Fraser River and 4) Hudson Bay Churchill-Saskatchewan-Nelson rivers).

(b) The distribution of lake whitefish hemoglobin phenotypes among four drainage basins in the the study area. Numbers refer to Figure 16 (a). Width of lines joining enclosed phenotype numbers indicates degree of affinity of population groups.

16a




YUKON
MACKENZIE
FRASER
HUDSON BAY
NIL
WF
13, 18
2,3,4,5,6,7,8,
10,11,12,13,14,15,17.
8,15
1,12
1,2,3,4,5,6,7,
8,9,10,11,12,13,
14,15,16,17,18.

16b

is obvious that a major association is suggested for populations in the Hudson Bay and Mackenzie Drainage basins, followed by considerably smaller associations between these two drainage basins and the Fraser River watershed, and even smaller associations with the Yukon River watershed. No hemoglobin phenotypes were observed to be common between the Fraser River and Yukon River drainage basins. Although the genetic and molecular basis of the hemoglobin phenotypes is yet to be spelled out, they are heritable (Table 5) and provide a certain measure of genetic evidence which adds weight to inferences based on the distributions of the other biochemical characters for which the genetics were elucidated.

MDH phenotypes display a similar geographic distribution to hemoglobin phenotypes and to the genetic characters. Pattern 1 (see Figure 10, MDH "Genetic Studies" section), in all probability a homozygote, is totally absent from Yukon populations sampled but is found at varying frequencies elsewhere. On the other hand, Yukon populations are characterized by very high frequencies of pattern 5, the other homozygote, according to the Bailey et al. (1970) model. Some Yukon populations also show variation in isozyme bands thought to represent forms of mitochondrial MDH.

This first part of the second section, "Population Studies", has shown the variation in frequencies of LDH heart type and G-3-PDH white muscle genes observed in populations of lake whitefish in Western Canada. Some additional information on similarities between populations has been provided by the occurrence of some phenotypes of MDH and hemoglobin. It has been demonstrated that

the observed distributions of population gene frequencies of the genetic characters probably has been the result of postglacial dispersal of lake whitefish from their glacial refugia rather than the result of a postglacial history of mutation and selection. It is the purpose of the second part of this section to demonstrate how this postglacial dispersal of lake whitefish took place.

Lake Whitefish Postglacial Dispersal and Distribution

The postglacial dispersal and distribution of lake whitefish will be examined from three viewpoints--one based on biochemical characters, one on the distribution of modal gillraker counts, and one on the distributions of other species. These viewpoints will be discussed individually and then combined in an overall statement concerning the present distribution of lake whitefish. Possible effects of selection on biochemical characters and gillraker number will be considered later.

1) Based on Biochemical Characters:

Biochemical characters used in this study, as outlined previously, are of two types--those with a known genetic basis (LDH, G-3-PDH A and B types) and those in which genetic bases are only implied (hemoglobin, MDH). If those characters of only implied genetic basis follow similar geographic patterns to those of known genetic basis, we can consider the case in question strengthened. Contrariwise, if the characters of only implied genetic basis contradict findings based on known genetic characters we can consider the case in question weakened but by no means shattered.

First let us consider the biochemical characters of known genetic basis.

a) Lactate Dehydrogenase:

The LDH $\underline{h}^b$ allele was found at varying frequency in all populations of lake whitefish outside of the Yukon River drainage, Alsek River drainage and Frances and Watson lakes of the Liard River headwaters. Of 151 fish from three lakes in the Yukon and Alsek drainages not one LDH $\underline{h}^b$ allele was discovered. The probability of discovering fish possessing this allele, by further sampling in the Yukon area, at this point was reduced to less than 5%. For this reason no further analyses were deemed necessary and Yukon and Alsek river populations of lake whitefish are considered to be homozygous for the LDH $\underline{h}^c$ allele. Outside of the Yukon and Alsek drainages, and Frances and Watson lakes, frequencies of LDH $\underline{h}^b$ are generally quite high (0.700-0.900) except for two headwater populations at opposite ends of the sampling area--Williams Lake, B.C. and Steeprock Lake, Manitoba which have frequencies of $\underline{h}^b$ of 0.136 and 0.135 respectively. As was pointed out earlier, with due respect to the heterogeneity of the geography over which sampling was carried out, no reasonable correlations were found between LDH $\underline{h}^b$ allele frequency and any geographic or climatic factor included in the analysis. On the other hand, frequencies of $\underline{h}^b$ allele exhibit a definite break in their distribution, concomitant with a geographic barrier, at the periphery of Yukon River watersheds. Frances and Watson lakes of the upper Liard River are allied to Yukon populations in their lack of the $\underline{h}^b$ allele. These lakes are on a tributary of the Liard, one branch of which drains Finlayson Lake

into Frances Lake. Finlayson Lake is only separated by a marsh (according to Mines and Technical Survey maps) from Pelly River of the Yukon system. Exchange of headwaters probably has taken place in this area and perhaps still does. Cache Lake, another Liard headwater but on the Nahanni River side of the mountains, is also spatially close to Yukon River tributaries but physically well separated from them and the lake's eastern affiliations are implied by a relatively high LDH $\underline{h}^b$ frequency (0.814). Thus on the basis of LDH $\underline{h}^b$ allele we might suggest a) lake whitefish from eastern refuges have not entered the Yukon watersheds b) because of the presence of $\underline{h}^c$ in non-Yukon populations lake whitefish probably exited from Yukon watersheds c) one exit has likely been via the upper Liard River drainages including Finlayson River and d) the distribution of the LDH $\underline{h}^b$ allele among lake whitefish populations in northwestern Canada probably has been influenced strongly by geography rather than other factors.

The distribution of LDH alleles among non-Yukon populations is in agreement with knowledge concerning water courses during deglaciation. In one way or another the localities of all non-Yukon populations have been connected via glacial lakes or their drainages, or by ice marginal water courses. The general picture of LDH $\underline{h}^b$ allele frequencies over the large area is one of uniformity, supporting the above contention. It is recognized here that a few populations have unusual (by comparison with most others) $\underline{h}^b$ frequencies--these will be discussed later.

b) Glycerol-3-Phosphate Dehydrogenase:

i) A subunit locus;

Two alleles $\underline{a}^1$ and $\underline{a}^2$ are seen in lake whitefish populations, the $\underline{a}^2$ allele generally being near a frequency 1.00. However, the $\underline{a}^1$ allele appears scattered over the whole sampling area at varying frequencies, reaching its maximum in a few Yukon populations. As outlined earlier the A^1 subunits may or may not be identical in Yukon and other populations or for that matter from area to area. If they are identical everywhere, then the allele producing the subunit either has developed by mutation independently and identically in different places, or has spread from a center of its highest frequency (Yukon) to other areas. The fact that frequencies of $\underline{a}^1$ decrease in all directions away from Squanga and Teenah lakes may support the latter idea. If the $\underline{a}^1$ allele did arise in and spread from a Yukon center, then we might expect on logical grounds, as discussed under LDH above, that the point of exit from Yukon drainages to the east would be via the upper Liard River. Frances Lake has an $\underline{a}^1$ allele frequency of 0.011 while Watson Lake showed no $\underline{a}^1$ allele present among 23 fish. Thus the exit of fish from the Yukon via upper Liard is neither confirmed nor refuted. We can conclude that the alleles of the G-3-PDH-$\underline{a}$ locus are of little value in connection with discerning routes of lake whitefish postglacial dispersal.

ii) B subunit locus;

The three glycerol-3-phosphate dehydrogenase $\underline{b}$ type alleles, $\underline{b}^1$, $\underline{b}^2$ and $\underline{b}^3$, provide probably the best single biochemical character

useful in the study of lake whitefish postglacial distribution. The $\underline{b}^2$ and $\underline{b}^3$ alleles occur in all populations analyzed, but frequencies vary considerably. Outside of the Yukon a positive correlation between $\underline{b}^2$ allele frequency and longitude is found, suggesting a cline with respect to this allele. Coincidentally this supposed cline falls generally along the probable route of fish movement during postglacial disersal. Since $\underline{b}^2$ frequencies are statistically higher in the western part of the sampling area one might suppose that the allele has spread from west to east. Coincident with this, frequencies of $\underline{b}^1$ allele are negatively correlated with longitude (and $\underline{b}^2$) suggesting that the two alleles might be mutually exclusive at extreme ends of the lake whitefish range. This is true in the west; lake whitefish populations in the Yukon River, upper Peace River, upper Athabasca and Fraser River drainages show no evidence of $\underline{b}^1$ allele (There is some indication from unpublished data that $\underline{b}^2$ is absent from a few eastern Canadian populations.). Frances and Watson lakes are of interest here; these populations also do not show the presence of $\underline{b}^1$ allele, strengthening the suggestion that they are closely related to Yukon drainage lake whitefish. The absence of $\underline{b}^1$ allele from Fraser River, upper Athabasca River, upper Peace River and Liard River headwater populations while at the same time being present in populations around Great Slave Lake suggests that $\underline{b}^1$ allele has spread westward from an eastern center either late or slowly during the postglacial dispersal of lake whitefish. The relative abundance of the $\underline{b}^1$ allele in Waterton Lakes whitefish supports this contention since this lake is reputed to have had a preglacial and glacial connection with the

upper Missouri River, a supposed refugium for fish during glaciation that was probably connected to eastern fish populations in a Mississippian refugium.

A similar scheme cannot be made for $\underline{b}^2$ allele because of its ubiquity in all populations sampled. However, one might explain the west-east cline in $\underline{b}^2$ frequencies as the result of a) spread during an earlier deglaciation or b) an early and rapid spread following Wisconsin glaciation. The second situation might have been possible since water connections between western and eastern drainages were best early in deglaciation and also because spread from west to east is mainly downstream. If the allele did arise in the Yukon or elsewhere in western North America one might expect it to have spread rather more rapidly eastward than an allele (e.g. $\underline{b}^1$) arising in the east spreading westward, particularly if that allele were to have arisen late in deglaciation.

c) Hemoglobin:

As indicated earlier, inferences about lake whitefish distribution based on variation of hemoglobins are to be taken with caution. Also it was stated, that if data from a character such as this supported lines of reasoning based on known genetic characters we might consider our case strengthened. Hemoglobin variation in lake whitefish falls in this category. Generally speaking, the frequencies of two postulated alleles (see hemoglobin genetics section) seem to indicate a break between Yukon and non-Yukon populations. One phenotype is in high frequency in populations sampled in the Yukon and relatively lower elsewhere. The variation is great

though, and it appears that selection may play an important role in determining relative frequencies of the supposed alleles. The system merits no further discussion than to say that observed variation in hemoglobin phenotypes does not contradict hypotheses made on the basis of the genetic characters. Finally, as shown earlier (Figure 16b), comparison of presence or absence of different hemoglobin phenotypes in the various drainages follows a pattern similar to that already suggested on the basis of other characters.

d) Malate Dehydrogenase:

Malate dehydrogenase phenotypes, as discussed earlier, appear to show a break between Yukon and non-Yukon populations of lake whitefish, but little else. This situation is not contradictory to what has been suggested on the basis of LDH and G-3-PDH geographic variation.

2) Based on Gillraker Counts:

Modal gillraker counts commonly have been used to characterize species and populations of whitefish. The shortcomings involved in the use of these characters were pointed out in the introduction. However, we can consider the structures to have an underlying genetic basis, but the various effects of selection could be quite large, particularly if gillraker number is a factor in food gathering, as many authors have intimated. For the moment, modal gillraker number will be considered a measure of population genotype. Again, keeping criteria set forth earlier regarding the use of characters of unknown genetic basis, we can consider the case describing lake whitefish zoogeography as strengthened if modal gillraker counts fit

the hypotheses, and weakened rather than shattered, if they do not. In this regard, modal gillraker counts are probably about as useful as hemoglobin phenotypes for characterizing lake whitefish populations, setting aside their convenience as a field character.

Modal gillraker counts (Figure 4) indicate that Yukon lake whitefish populations are set apart from almost all nearby non-Yukon populations. There are indications that the upper Liard River populations are unlike Yukon populations and that populations in the lower Mackenzie River are allied to Yukon rather than non-Yukon populations. Also, a few locations in the Yukon and Alaska hold populations in which modal gillrakers counts are a little higher than most other populations in the area. A small amount of variation is to be expected. However, two upper Liard River populations seem to indicate some gene flow from eastern populations. No explanation is available for this fact save perhaps that local selection may have been at work there--in short, these two lakes do not fit the scheme developed on the basis of biochemical characters. The presence of low modal gillraker counts in the lower Mackenzie River populations can be explained on the basis of coastal dispersal from Alaska and thence up Mackenzie River. This is the route suggested for the entry of Coregonus nasus into the Mackenzie River and probably also the route for lake whitefish in that area (McPhail and Lindsey, 1970). Populations in the upper Peace River and the upper Fraser river are closely similar to populations in most of central Canada. Also, Waterton Lakes lake whitefish are similar in modal gillraker number to central Canadian populations. In general, data on gillraker numbers support

hypotheses generated on the basis of biochemical characters regarding separation of Yukon whitefish populations from those in the rest of Western and central Canada. It was impossible to obtain samples for biochemical analysis from lower Mackenzie River populations for direct comparisons with gillraker data from there. The presence of populations with two modes of gillraker number in some Yukon lakes (Squanga, Teenah and possibly Dezadeash) is of note; these populatins will be discussed further later.

3) Based on the distribution of other Fish Species:

The present distributions of a number of fish species provide a considerable insight into the major postglacial dispersal routes taken by fish from the different glacial refuges. Evidence for possible postglacial dispersal routes of lake whitefish can be gained in this way. This type of evidence is not the same as that set forth previously, since the extent to which ecological factors are involved in the limitation of the distribution of the various species considered is unknown. On the other hand, where the distribution of a species crosses a watershed divide in a place consistent with known geological history we may assume that the crossing took place as a result of an historical geological event; the spread of a species, once it has crossed a divide into a "new" watershed is where ecological factors play their role. Of course, a species introduced into a new watershed conceivably could be eliminated from it secondarily. The information to be presented here is summarized from McPhail and Lindsey (1970), the most recent information available on the distribution of freshwater fishes in northwestern North America. Since these authors have provided

evidence that lake whitefish probably survived glaciation in only the Bering and Mississippi refugia in Western Canada, our discussion will involve mainly the dispersal of fishes from these two refugia.

Ten species of fishes are thought to have survived glaciation in only the Bering refugium. Of these, six species (Lampetra japonica, Stenodus leucichthys, Coregonus sardinella, Coregonus autumnalis, Coregonus laurettae and Hypomesus olidus) commonly enter the sea and have dispersed along coastal areas; L. japonica, C. laurettae and H. olidus have dispersed both east along the Arctic coast and south into the Gulf of Alaska; the other three have dispersed only eastward along the Arctic coast. Coregonus nasus and Coregonus pidschian (the name applied by McPhail and Lindsey (1970) to lake whitefish with low counts of gillrakers found in Yukon and Alaska) are tolerant of brackish waters and have also dispersed eastward along the Arctic coast. Of these eight species that have dispersed from the Bering refugium eastward along the Arctic coast, C. nasus, C. sardinella and C. autumnalis have been recorded up the Mackenzie River as far as Camsell Bend; L. japonica and S. leucichthys as far as Great Slave Lake and H. olidus as far as Great Bear Lake. C. laurettae and C. pidschian are believed to occur in only the lower parts of the Mackenzie River or in coastal areas. The ninth species found only in the Bering refugium, Dallia pectoralis is totally restricted to freshwater and has enlarged its distribution postglacially little if at all. A final species of consideration is another lake whitefish, Coregonus nelsoni; at present it is uncertain if this is a valid species at all, if

so it apparently has not expanded its range postglacially (McPhail and Lindsey, 1970). In the interior of the Yukon, Coregonus pidschian (or possibly C. nelsoni) is believed to have entered the Alsek River system (Aishihik and Dezadeash lakes) by exchange of waters between this river and the Yukon River. As mentioned in the introduction, the area of the Alsek River drainage is subject to glacial action even to the present time; at least once, perhaps several times, waters now running south via the Alsek River into the Gulf of Alaska have been dammed by glaciers and forced to run northwest into the Bering Sea via the Yukon River. The Bering morphological forms of three other species, Thymallus arcticus, Prosopium cylindraceum and Lota lota, are believed to have entered the Alsek River in the same manner.

Eighteen species of fishes that have entered northwestern North American watersheds are derived from a Mississippi refugium (including upper Missouri River). The northern distributions of all of these species except two, Catostomus commersoni and Percopsis omiscomaycus, are restricted to the Mackenzie River system. C. commersoni is also present in the upper Dubawnt River of Keewatin District, while P. omiscomaycus is found also in the Porcupine River of the Yukon River system; this latter species will be considered again later. The interesting point to be made here is that several species (Platygobio gracilis, Notropis atherinoides, Notropis hudsonius, Percopsis omiscomaycus, Cottus ricei, Pfrille neogaea, Stizostedion vitreum and possibly Hiodon alosoides and Catostomus commersoni) have been unable to invade the upper Liard River (above Liard Canyon) in Yukon Territory. Thus the Liard Canyon has provided a

barrier through which many southern fishes have been unable to pass. The reverse, of course, would not necessarily be true. The canyon on the Peace River has posed a similar barrier to the movements of fishes; seven of the above species were unable to ascend to the upper reaches of the Peace River. Thus of seventeen species of fishes emigrating from the Mississippi refugium, nine species entered the Liard River as far as the bottom of Liard Canyon. It does not seem likely that the eighteenth species and the tenth to enter Liard River, Coregonus clupeaformis, a lake-adapted fish, would have been the only Mississippi emigrant to ascend Liard Canyon and reach Liard River headwaters. The Peace River canyon probably did not pose such a strong barrier because of glacial Lake Peace; this point will be considered later.

More evidence for a one way movement of fish faunas from Yukon drainages into central Canada comes from a consideration of several species of fishes that endured glaciation in more than one refugium.

Two species (Osmerus eperlanus and Salvelinus alpinus) survived glaciation in both the Bering and Atlantic refugia. Both species show two morphological forms and in both cases the Bering form has spread eastward along the Arctic coast.

Five species (Prosopium cylindraceum, Thymallus arcticus, Salvelinus namaycush, Esox lucius and Pungitius pungitius) survived glaciation in the Bering and Mississippi refugia. E. lucius and T. arcticus each have a Bering form which is mainly restricted to the area of the Bering refugium, but in both cases this form has spread southward into the Liard River and some Pacific drainages

(e.g. Taku River). The Mississippi forms of these species are present in the Mackenzie River system and eastern drainages. P. cylindraceum also exists in two forms; in this instance the Bering form has not only spread southward into the Liard River and Pacific drainages as far south as Taku River, but has also spread eastward into the Mackenzie River system and from there southeastward as far as the Churchill River system. The Mississippi form of this species has spread only into northern Ontario at the western extent of its range, leaving a gap in the distribution of the species in northeastern Manitoba. P. pungitius has two forms also, the Bering form having spread eastward along the Arctic coast possibly as far as the Atlantic Ocean and southward into the Gulf of Alaska. No clearly differentiated morphological forms of S. namaycush have been identified to date.

Four species (Prosopium coulteri, Catostomus catostomus, Lota lota and Cottus cognatus) may have survived glaciation in the Bering, Mississippi and Pacific refugia. Two species, L. lota and C. cognatus have distinct Bering and Mississippi forms and an intermediate form in the area of the Pacific refugium. The Bering form of L. lota has spread into the Alsek River and into the upper Liard River; throughout the Mackenzie system this form intergrades with a Mississippi form common in all major Barren Ground rivers. C. cognatus shows a similar picture, except that the Bering form has spread further south, into Pacific drainages, intergrades with its Mississippi counterpart further to the east and exhibits a third

form in most B.C. locations which is intermediate to the two "pure" forms. C. catostomus similarly has two forms, the Bering form spreading out of the Bering refugium into the upper Liard River and possibly into the Peace and Fraser rivers. The Mississippi form of this species is found in the Mackenzie River system and Barren Ground rivers. The presence of relict forms of C. catostomus in Western Washington provides evidence for its survival in the Pacific refugium as well. The fourth species in this group, P. coulteri, has a puzzling array of forms suggesting survival in several refugia, likely the Bering, Mississippi, Missouri and Pacific. The existence of a distinct form of pygmy whitefish in Waterton Lakes (Lindsey and Franzin, 1972) coupled with a native lake trout population and early records of lake trout in some northern Montana lakes (Schultz, 1941) suggests lake whitefish native to Waterton Lakes may have survived glaciation in the upper Missouri. This does not, however, exclude the possibility of entry of lake whitefish from the northeast via the South Saskatchewan River.

Salvelinus malma, the Dolly Varden, is thought to have survived glaciation in the Bering and Pacific refugia. Two forms are seen in this fish but in this case the Bering form is restricted, while the Pacific form is thought to have spread northward into Liard, Peace and Athabasca headwaters, and into the headwaters of the Yukon and Tanana rivers. Another species, Prosopium williamsoni, having survived glaciation in the Pacific refugium, has also spread northward into the upper Liard River and into the lower Mackenzie River (C.C. Lindsey, Pers. Com). The Dolly Varden is able to enter the sea

and conceivably could have entered the Liard headwaters via the Pacific Ocean and the Yukon River. P. williamsoni, however must have entered the Liard directly somehow--it is adapted to life in mountain streams and thus may have ascended the Liard Canyon. Some Pacific coast river headwaters (e.g. Stikine) containing both S. malma and P. williamsoni are in close juxtaposition with tributaries of the upper Liard River. In a mountainous area such as this, local glacial activity could have enabled headwater transfer of these two species; certainly some similar such events must have allowed the southward movement of Bering forms of other species mentioned previously.

Only two other southern species have managed to invade Yukon watersheds--Percopsis omiscomaycus and Couesius plumbeus. McPhail and Lindsey (1970) suggest that both species have invaded the Yukon River by relatively recent headwater transfer from the Peel River of Mackenzie River system. The fact that the morphological form of each species found in the Yukon headwater where they occur is similar to that found in the Mackenzie system and the rest of their southern and eastern ranges seems to be good evidence of this. Lack of data on lake whitefish populations in the area in question prevents even conjecture as to whether a similar headwater transfer of lake whitefish might have occurred.

Summarizing, the bulk of evidence from the present distribution of a number of fish species and morphological forms of several other fishes supports the contention based on biochemical evidence that gene flow in lake whitefish has been unidirectionally

out of the Yukon River system and the Bering refugium. The fact that several species, probably as adaptable and as capable swimmers as lake whitefish, have been unable to ascend Liard Canyon suggests that lake whitefish have been unable to get into the Yukon by headwater transfer from Liard River. Conversely, the fact that several species and Bering forms of species have entered the upper Liard from the Yukon suggests that lake whitefish found in Frances and Watson lakes (and probably all other populations in the area as well) are derived from Yukon populations, thus accounting for their biochemical relatedness to Yukon lake whitefish. This link provides also a significant avenue for gene flow out of the Bering refugium into central Canada. That this gene flow could be significant and have far reaching effects is borne out by the distance emigrated by the Bering form of round whitefish discussed previously. The presence of lake whitefish in upper Peace and Fraser river watersheds indicates that this species was able to ascend the Peace Canyon and enter Fraser River, probably during the time when these drainages were connected and when water levels in Peace River would have been high (10-12,000 B.P.) due to the presence of glacial lakes Peace and Prince George. Thus, one might conclude that lake whitefish were among the early arriving fishes in that area since several other species have been unable to ascend Peace Canyon, perhaps due to a later arrival.

Chronology

It is possible now to erect a tentative chronology for the

postglacial dispersal and distribution of lake whitefish over central Canada. Although some geological dates will be suggested, most dates which follow may be regarded in a relative sense only.

There are probably three main stages in the chronology of lake whitefish Wisconsin history. These are a) glacial, b) early deglaciation and c) postglacial to recent. During the glacial period, until about 14,000-16,000 B.P., lake whitefish survived in the Bering and Mississippi refugia. We might envision their genotypes, in terms of the proteins for which the genetics have been determined, and the sequence of events as follows: 1) Glacial State (Figure 17): a) Bering refugium--LDH-$\underline{h}^c$ allele only, G-3-PDH-$\underline{b}^2$ and $\underline{b}^3$ alleles, $\underline{b}^1$ allele not present, G-3-PDH-$\underline{a}^1$ and $\underline{a}^2$ alleles present, $\underline{a}^1$ perhaps only in a few isolated headwater populations; b) Mississippi (and Missouri) refugium--LDH-$\underline{h}^b$ allele only, G-3-PDH-$\underline{b}^3$ allele, plus possibly $\underline{b}^2$ allele, $\underline{a}^2$ allele only. c) Pacific refugium--no lake whitefish present as evidenced by their absence in suitable cool deep lakes in Columbia River headwaters (lake whitefish have been successfully introduced to Kootenay Lake) 2) Early Deglaciation (Figure 18): 10-12,000 B.P. Fish spread rapidly out of refugia, intermixing in central Canada. Lake whitefish carrying LDH-$\underline{h}^b$ and $\underline{h}^c$, G-3-PDH-$\underline{b}^2$ and $\underline{b}^3$, $\underline{a}^2$ only, entered upper Peace River and Fraser River watersheds via glacial lakes Peace and Prince George, and also headwaters of Athabasca and North Saskatchewan rivers. As deglaciation continued water levels in Lake Peace lowered, fish could no longer ascend Peace Canyon or easily enter headwaters of western rivers. Movement of fishes down Mackenzie River began; lake whitefish carrying $\underline{a}^1$ allele of G-3-PDH

Figure 17 Hypothetical distribution of alleles of biochemical characters considered in the present study during the maximum of Wisconsin glaciation. Numbers refer to glacial refugia: 1) Bering, 2) Pacific , 3a, 3b) Missouri-Mississippi and 4) Atlantic.

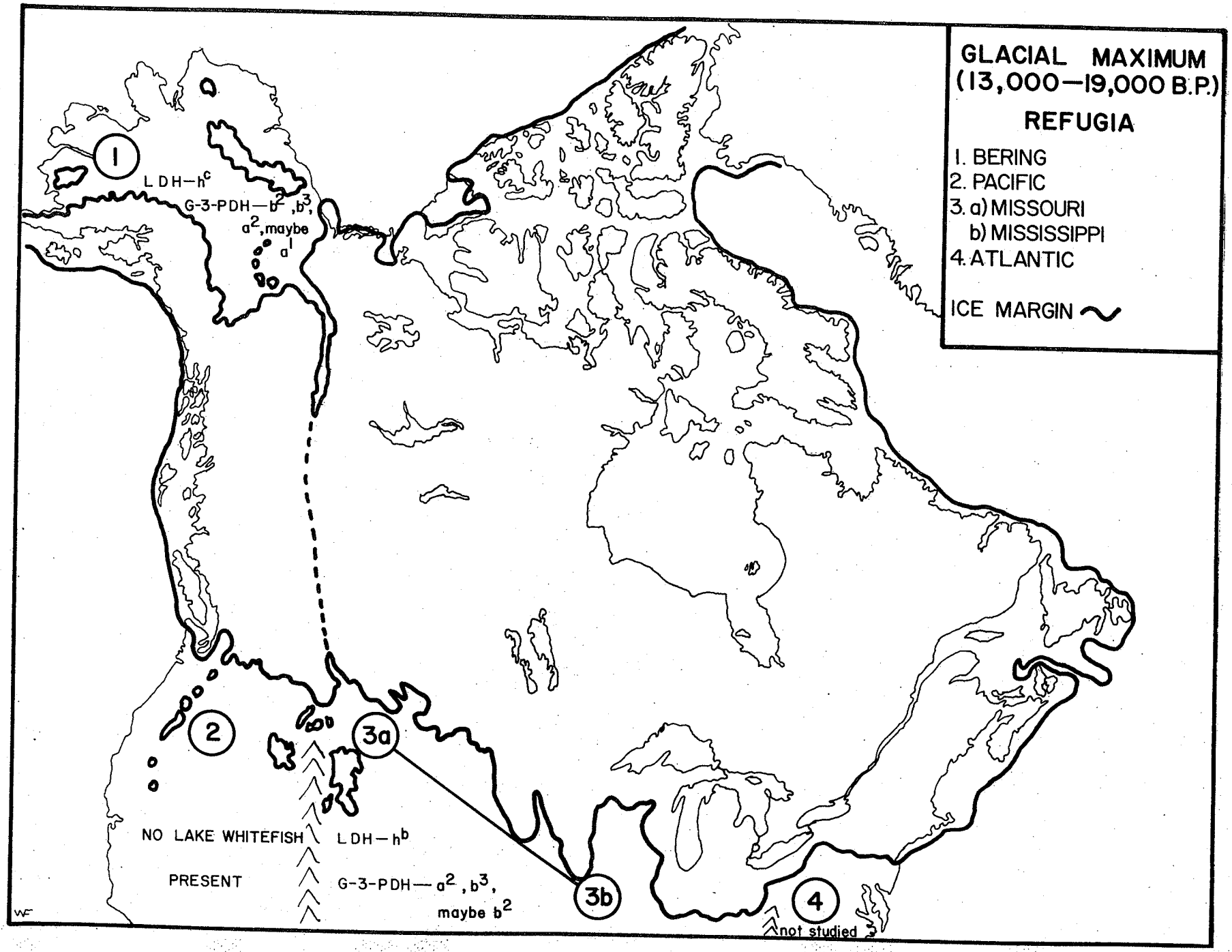
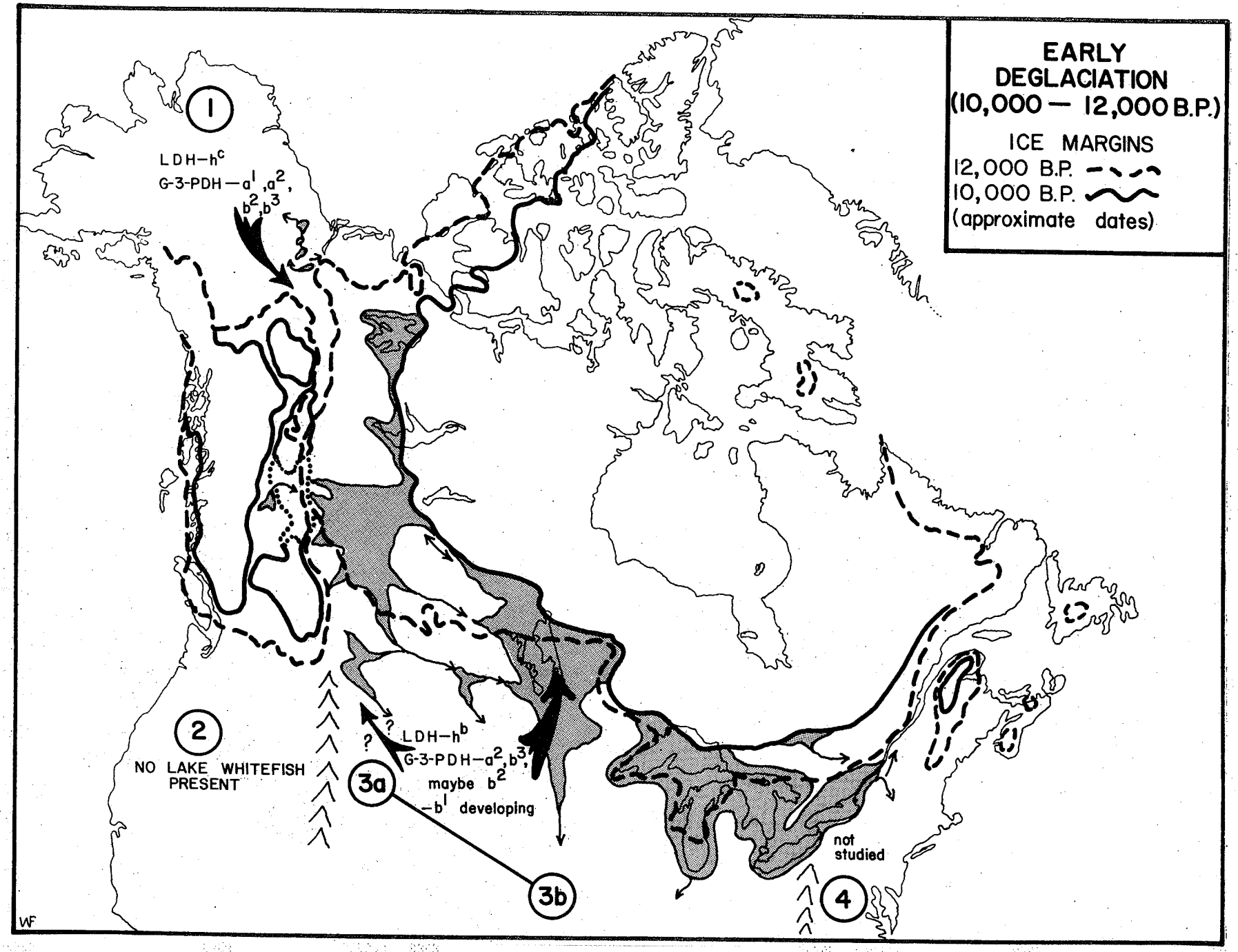

GLACIAL MAXIMUM
(13,000—19,000 B.P.)
REFUGIA
1. BERING
2. PACIFIC
3. a) MISSOURI
b) MISSISSIPPI
4. ATLANTIC
ICE MARGIN
1
LDH—h^c
G-3-PDH—b^2, b^3, a^2, maybe a^1
2
3a
3b
4
not studied
NO LAKE WHITEFISH PRESENT
LDH—h^b
G-3-PDH—a^2, b^3, maybe b^2

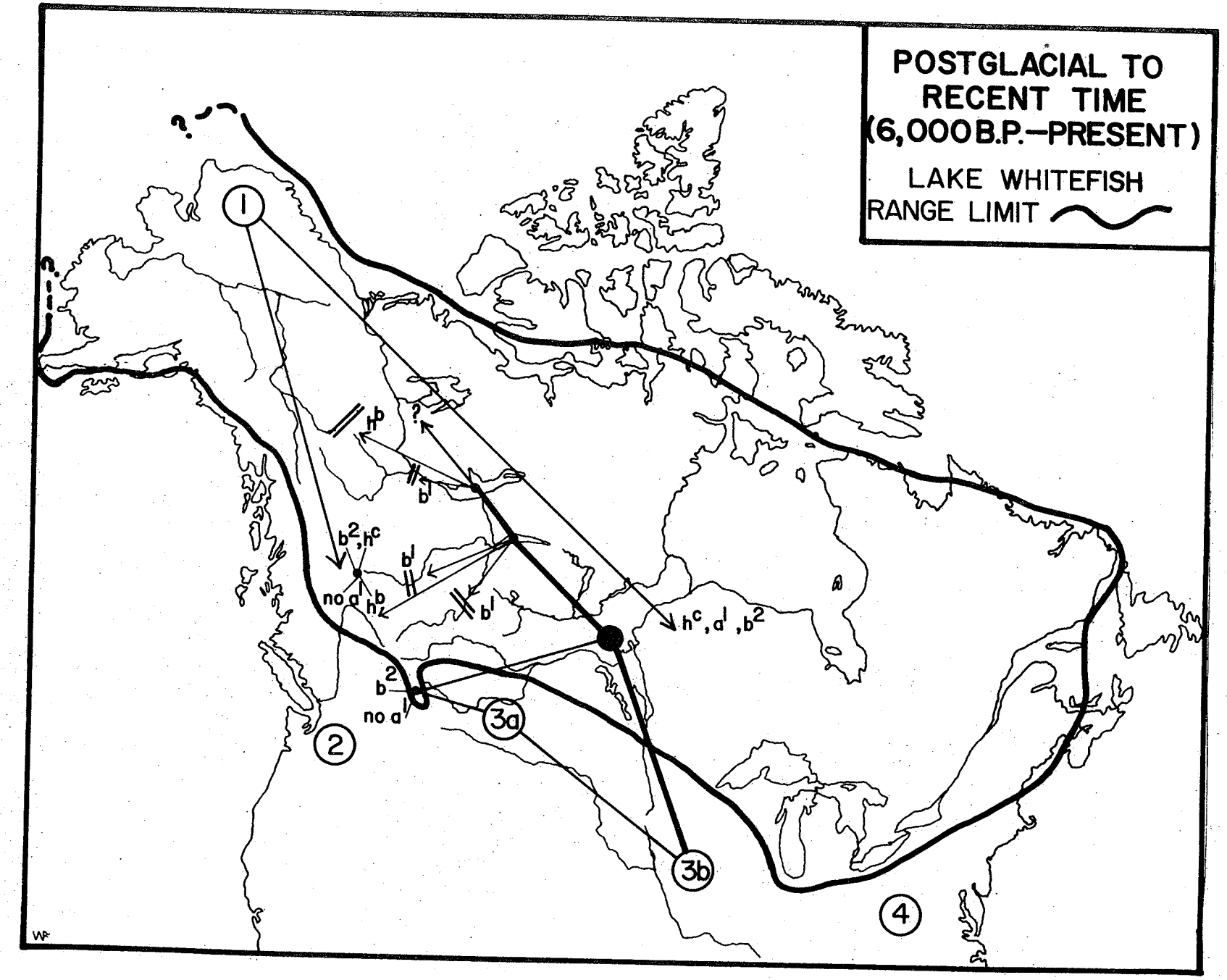
Figure 18 Hypothetical dispersal of lake whitefish early in deglaciation as indicated by biochemical characters. Numbers refer to refugia indicated in Figure 17.


EARLY
DEGLACIATION
(10,000 — 12,000 B.P.)
ICE MARGINS
12,000 B.P.
10,000 B.P.
(approximate dates)
1
LDH—h^c
G-3-PDH—a^1, a^2, b^2, b^3
2
NO LAKE WHITEFISH PRESENT
3a
?
?
LDH—h^b
G-3-PDH—a^2, b^3; maybe b^2 —b^1 developing
3b
not studied
4
WF

began "leaking" out of Yukon via Liard River, lake whitefish carrying G-3-PDH-$\underline{b}^1$ allele, developed late, in the Mississippi refugium, entered central Canada spreading this allele rapidly northwest via Lake Agassiz, Lake Tyrrell and/or Lake McConnell. G-3-PDH-$\underline{a}^1$ allele spread slowly eastward. The G-3-PDH-$\underline{b}^1$ allele may have developed in the east (where it is in high frequency in some present populations) and spread to central Canada via the Great Lakes--Lake Agassiz connections, but the high frequency (0.63) of this allele in Waterton Lakes would argue against this, suggesting instead a Mississippi-Missouri origin for the $\underline{b}^1$ allele. 3) Postglacial to Recent (Figure 19): The lake whitefish distribution was becoming discontinuous; headwater populations were becoming isolated. G-3-PDH-a^1 allele continued spreading slowly eastward, still "leaking" out of Yukon watersheds from time to time via the Liard River.

In this manner we have arrived at the distribution of lake whitefish population gene frequencies that we see today. The break between lake whitefish populations in the Yukon and central Canada and B.C. is a result of the admixture of a large portion of Mississippi stock with a small portion of Bering stock. Subsequent to the initial mixing of the two stocks, lake whitefish entered upper Peace and Fraser River watershed as well as several other western headwaters. These populations were then isolated, and in central Canada the mixing of the two stocks probably has continued to some degree to present times. Gene flow out of the Bering refugium and Yukon has been similar to water falling over a cliff--no gene flow has occurred in the opposite direction.

Figure 19. Present distribution of biochemical character "indicators" and supposed routes by which the main flow of lake whitefish postglacial dispersal has occurred. Numbers refer to refugia indicated in Figure 17.


POSTGLACIAL TO
RECENT TIME
(6,000 B.P.—PRESENT)
LAKE WHITEFISH
RANGE LIMIT
1
2
3a
3b
4
?
?
?
h^b
b^1
b^2, h^c
b^1
no a^1, h^b
b^1
h^c, a^1, b^2
b^2
no a^1
WA

Selection

There have been two stages in the Wisconsin-Recent history of lake whitefish populations in northwestern North America. The first lasted 40,000 years or more, during which lake whitefish were isolated into two main glacial refugia. The second stage has lasted 5,000-10,000 years, the period since the disappearance of the major ice sheets, during which lake whitefish have distributed themselves over most of northern North America.

During long isolation in the two widely-separated refugia, lake whitefish must have been subjected to different sets of conditions of day length, growing season, winter ice cover, mean annual temperature etc., factors producing differential selection in the two areas. Selection in two geographically well-separated refugia probably led to the development of two separate stocks of lake whitefish, both in terms of biochemical characters such as those used in the present study and also in terms of morphometric characters such as gillraker number. In this manner, selection could have produced lake whitefish population genotypes optimal to the conditions prevalent in each refugium.

Subsequent to disappearance of the major ice sheets, lake whitefish stocks in the two refugia were provided with ample opportunity, through late-glacial and postglacial watercourses and lakes, to distribute themselves over most of northern North America. This process has been fairly rapid compared to the period of isolation, and in a small way probably continues to present times. Most of central Canada has been criss-crossed with interconnecting watercourses, apparently allowing thorough intermixing of the two presumed

stocks of lake whitefish. The rather uniform distribution of the biochemical genetic characters used in this study, together with data on distribution of population modes of gillraker number in central Canadian lake whitefish supports this contention. For most central Canadian lake whitefish populations, postglacial selection seems to have been unimportant. This does not mean that in terms of the characters studied no selection has been or is acting on central Canadian lake whitefish, but rather that the intermixing of populations over wide areas of central Canada during the last few thousand years probably has been sufficient to wipe out the effects of selection in any given location. Probably only in a few long-and well-isolated headwaters has there been sufficient opportunity for selection to become important. Thus lake whitefish populations from peripheral drainages such as the headwaters of the Saskatchewan, Peace and Athabasca rivers, small isolated headwater lakes (e.g. Clear Lake and Steeprock Lake in Manitoba and Cache Lake in Northwest Territories) and the early-isolated Fraser River drainage show gene frequencies different from those of the main waterways of Central Canada. Just such populations are subject to chance, in terms of colonization, genetic drift in small populations and selection (e.g. in periods of climatic extreme). In some of these lakes e.g. Clear Lake and Steeprock Lake, deviations in gene frequencies from populations in lakes in the surrounding area are seen.

The Yukon populations of lake whitefish are set apart from populations in the rest of northern North America both in terms of the biochemical characters used in this study and gillraker

numbers. The coincident break in the distributions of each of the biochemical characters, together with a definite change in the distribution of population modes of gillraker number between Yukon and non-Yukon lake whitefish populations seems to provide strong, albeit circumstantial, evidence that the division of lake whitefish populations into the two groups is a result of divergence in the two refugia followed by postglacial dispersal. Such a division, indicated by several independent characters, is inconsistent with hypotheses based on postglacial selection. Similar arguments can be made to explain differences between B.C. and central Canadian lake whitefish populations though perhaps less forcefully.

Two lake whitefish populations were noted earlier for their significant deviations from Hardy-Weinberg expectations; Fox Lake, Yukon Territory showed a deficiency of heterozygotes of G-3-PDH B type phenotypes, while Lac la Hache, B.C. showed a deficiency of LDH heterozygotes. Koehn et al. (1971) have reviewed a number of possible explanations for this type of observation. The present study does not include enough detailed information about population structure to choose one of these explanations but heterogeneity in the sample may be the best choice. Since lake whitefish probably move around in more or less stable groups and gillnetting was carried out usually over a twelve hour period, it is conceivable that a few or several genetically slightly different schools or groups of fish could have been sampled in some lakes.

The effects of selection on gillraker number in lake whitefish

have received considerable attention in the literature and it is beyond the scope of the present study to undertake a detailed consideration of this problem. However, there are two and perhaps three Yukon lakes (Squanga, Teenah and probably Dezadeash) in which lake whitefish populations exhibit a bimodal distribution of gillraker numbers. These populations offer the opportunity for an interesting future study of the interrelationship of biochemical genetic characters and gillraker numbers in the context of single populations.

SUMMARY AND CONCLUSIONS

1) Genetic Studies

Results of a previous study (Franzin, MS 1970, Clayton and Franzin, 1970) established the genetics of electrophoretic phenotypes of LDH from red muscle of lake whitefish and formed the basis of the use of LDH population gene frequencies in the present study.

Electrophoretic phenotypes of G-3-PDH from lake whitefish muscle, through data from two breeding experiments (1968, 1970), were determined to be the result of the action of two independent loci, an a locus having two non-dominant alleles a^1 and a^2, and a b locus having three non-dominant alleles b^1, b^2 and b^3. Combinations of the products of the two loci resulted in a predicted total of eighteen phenotypes, fifteen of which were observed among wild populations.

The 1970 breeding experiment included an analysis of the inheritance of five observed types of electrophoretic phenotypes of lake whitefish muscle supernatant MDH. Most of the breeding data fit a model proposed by Bailey et al. (1970) to explain electrophoretic phenotypes of king salmon and rainbow trout muscle supernatant MDH, but in a number of matings expected ratios of progeny phenotypes were not obtained. Also some lake whitefish exhibited different phenotypes for red and white muscle. Because of these inconsistencies, no definite conclusion was reached on the inheritance of electrophoretic phenotypes of lake whitefish muscle supernatant MDH.

Eighteen different electrophoretic hemoglobin phenotypes were observed among wild populations of lake whitefish. Data from

the 1968 and 1970 breeding experiments could not be interpreted on a genetic and molecular basis because of lack of knowledge on: a) the number of genes controlling phenotypes and b) the basic structural composition of individual bands in the electrophoretic phenotypes.

2) Population Studies

Lake whitefish samples of from 14 to 251 individuals were obtained from each of 38 lakes spanning the area from the Manitoba-Ontario border west to central B.C. and western Yukon Territory. Electrophoretic analysis for G-3-PDH and MDH phenotypes was carried out for all samples; LDH phenotypes were determined for all but some of the Yukon populations (determined to be all the same, statistically). Hemoglobin phenotypes were also determined only for some of the populations. Correlation coefficients were calculated for relationships among the biochemical characters of defined genetic basis and a few geographic and climatic factors. The data were then interpreted in the context of available knowledge of the events of the Wisconsin glacial period. With the addition of knowledge obtained from the literature about the distributions both of gillraker number and other freshwater fish species, a scheme was devised to describe the postglacial dispersal of lake whitefish from their two main glacial refugia, the Bering and Mississippi refugia. Through a synthesis of geological and biological information, a tentative chronology for the postglacial dispersal of lake whitefish was developed. The coincident break between Yukon and non-Yukon populations in distributions of gillraker counts and of biochemical

characters, which are rather uniform over most of central Canada, was considered evidence that the variation in these characters is the result of divergence (at least partly due to differential selection) in the two main refugia, followed by postglacial disersal, rather than a result of postglacial selection.

A brief history of the geology of the Wisconsin glaciation is presented as Appendix 1.

REFERENCES

Bailey, G.S., G.T. Cocks, and A.C. Wilson. 1969. Gene duplication in fishes: Malate dehydrogenases of salmon and trout. Biochem. Biophys. Res. Commun. 34: 605-612.

Bailey, G.S., and A.C. Wilson. 1968. Homologies between isoenzymes of fishes and those of higher vertebrates. J. Biol. Chem. 243 (22): 5843-5853.

Bailey, G.S., A.C. Wilson, J.E. Halver and C.L. Johnson. 1970. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. Biochemical, immunological and genetic studies. J. Biol. Chem. 245: 5927-5940.

Benesch, R. and R.E. Benesch. 1969. Intracellular organic phosphates as regulators of oxygen release by hemoglobin. Nature 221:618-622.

Broesmer, R.W. and R.R. Marquardt. 1966. Insect extramitochondrial glycerophosphate dehydrogenase. II. Enzymic properties and amino acid composition of the enzyme from honey bee (Apis mellifera) thoraces. Biochim. Biophys. Acta 128: 464-473.

Clayton, J.W. Personal Communication.

Clayton, J.W. and W.G. Franzin. 1970. Genetics of multiple lactate dehydrogenase isozymes in muscle tissue of lake whitefish (Coregonus clupeaformis). J. Fish. Res. Bd. Canada 27: 1115-1121.

Clayton, J.W., W.G. Franzin, and D.N. Tretiak. 1973. Genetics of glycerol-3-phosphate dehydrogenase isozymes in white muscle of lake whitefish (Coregonus clupeaformis). J. Fish. Res. Bd. Can. 30: 187-193.

Clayton, J.W., and D.N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Can. 29: 1169-1172.

Clayton, J.W., D.N. Tretiak and A.H. Kooyman. 1971. Genetics of multiple malate dehydrogenase isozymes in skeletal muscle of walleye (Stizostedion vitreum vitreum). J. Fish. Res. Bd. Can. 28: 1005-1008.

Craig, B.G. 1965. Glacial Lake McConnell, and the surficial geology of parts of Slave River and Redstone River map-areas, District of Mackenzie. Geol. Surv. Can. Bull. 122.

Efremov, G.D., T.H.J. Huisman, Linda L. Smith, J.B. Wilson, Janice L. Kitchens, Ruth N. Wrightstone and H.R. Adams. 1969. Hemoglobin Richmond, a human hemoglobin which forms asymmetric hybrids with other hemoglobins. J. Biol. Chem. 244: 6105-6116.

Elson, J.A. 1967. Geology of Glacial Lake Agassiz: In Life, Land and Water; Proc. of 1966 Conference on Environmental Studies of the Glacial Lake Agassiz Region. (Ed.). W.J. Mayer-Oakes. Univ. Manitoba Press, Winnipeg, Man., Can. pp. 36-95.

Engel, W., J. Schmidtke and U. Wolf. 1971. Genetic variation of α-glycerophosphate dehydrogenase isoenzymes in clupeoid and salmonoid fish. Experentia (Basel) 27: 1489-1491.

Flint, R.F. 1971. Glacial and Quaternary Geology. John Wiley and Sons, Inc., New York. 892 p.

Franzin, W.G. MS, 1970. Lactate dehydrogenase and hemoglobin variants in lake whitefish, Coregonus clupeaformis (Mitchill). M. Sc. Thesis. Univ. Manitoba, Zool. Dept., Winnipeg, Man., Can. 53 p.

Gillen, R.G., and A. Riggs. 1972. Structure and function of the hemoglobins of the carp, Cyprinus carpio. J. Biol. Chem 247: 6039-6046.

Holmes, R.S., and C.L. Markert. 1969. Immunochemical homologies among subunits of trout lactate dehydrogenase isozymes. Proc. Nat. Acad. Sci. U.S.A. 64: 205-210.

Hughes, O.L. Personal Communication.

Hughes, O.L. 1972. Surficial geology of northern Yukon Territory and northwestern District of Mackenzie, Northwest Territories. Geol. Surv. of Can. Paper 69-36.

Hunt, W.G., and R.K. Selander. 1973. Biochemical genetics of hybridization in European house mice. Heredity 31: 11-33.

Johnson, A.G., F.M. Utter, and H.O. Hodgins. 1970. Electrophoretic variants of L-alpha-glycerophosphate dehydrogenase in Pacific ocean perch (Sebastodes alutus). J. Fish. Res. Bd. Can. 27: 943-945.

Kaplan, N.O., and M.M. Ciotti. 1961. Evolution and differentiation of dehydrogenases. Ann. N.Y. Acad. Sci. 94: 701-720.

Kindle, E.D. 1953. Dezadeash map-area, Yukon Territory. Geol. Surv. Can. Mem. 268.

Kindle, E.M. 1929. The Geological Story of Jasper National Park, Canada. Dept. of Int., Nat. Parks of Can., Ottawa, Can. 48 p.

Kitto, G.B. 1966. The comparative enzymology of malate dehydrogenases. Ph.D. Thesis, Brandeis Univ., Waltham, Mass. 381 p.

Klose, J., U. Wolf, H. Hitzeroth, H. Ritter, N.B. Atkin, and S. Ohno. 1968. Duplication of the LDH gene loci by polyploidization in the fish order Clupeiformes. Humangenetik 5: 190-198.

Koehn, R.K., J.E. Perez, and R.B. Merritt. 1971. Esterase enzyme function and genetical structure of populations of the freshwater fish, Notropis stramineus. Amer. Nat. 105: 51-69.

Li, S.L., S. Tomita, and A. Riggs. 1972. The hemoglobins of the Pacific hagfish, Eptatretus stoutii. I. Isolation, characterization and oxygen equilibria. Biochim. Biophys. Acta 278: 344-354.

Lindsey, C.C. Personal Communication.

Lindsey, C.C., J.W. Clayton, and W.G. Franzin. 1970. Zoogeographic problems and protein variation in the Coregonus clupeaformis whitefish species complex. In Biology of Coregonid Fishes. (Ed's) C.C. Lindsey and C.S. Woods. Univ. Manitoba Press, Winnipeg, Man., Can. pp. 127-147.

Lindsey, C.C., and W.G. Franzin. 1972. New complexities in zoogeography and taxonomy of the pygmy whitefish (Prosopium coulteri). J. Fish. Res. Bd. Can. 29: 1772-1775.

Marquardt, R.R. and R.W. Broesmer. 1966. Insect extramitochondrial glycerophosphate dehydrogenase. I. Crystallization and physical properties of the enzyme from honey bee (Apis mellifera) thoraces. Biochim. Biophys. Acta 128: 454-463.

Massaro, E.J., and C.L. Markert. 1968. Isozyme patterns of Salmonid fishes: Evidence for multiple cistrons for lactate dehydrogenase polypeptides. J. Exp. Zool. 168: 223-238.

Mathews, W.H. 1963. Quaternary stratigraphy and geomorphology of the Fort St. John area, northeastern British Columbia. B.C. Dept. of Mines Petrol. Res.

McCabe, M.M., D.M. Dean, and C.S. Olson. 1970. Multiple forms of 6-phosphogluconate dehydrogenase and alpha-glycerophosphate dehydrogenase in the skipjack tuna, Katsuwonus pelamis. Comp. Biochem. Physiol. 34: 755-757.

McPhail, J.D. and C.C. Lindsey. 1970. Freshwater Fishes of Northwestern Canada and Alaska. Fish. Res. Bd. Can. Bull. 173. 381 p.

Metcalfe, A.L. 1966. Fishes of the Kansas River System in relation to zoogeography of the Great Plains. Univ. Kansas Publ., Mus. Nat. Hist. 17: 23-189.

Mulligan, R. 1963. Geology of the Teslin map-area, Yukon Territory. Geol. Surv. Can. Mem. 326.

Murphy, W.H., G.B. Kitto, J. Everse, and N.O. Kaplan. 1967. Malate dehydrogenase. I. A survey of molecular size measured by gel filtration. Biochemistry 6: 603-610.

Numachi, K. 1970. Polymorphism of malate dehydrogenase and genetic structure of juvenile population in saury (Cololabis saira). Bull. Jap. Soc. Sci. Fish. 36: 1235-1241.

Numachi, K., Y. Matsumiya and R. Sato. 1972. Duplicate genetic loci and variant forms of malate dehydrogenase in chum salmon and rainbow trout. Bull. Jap. Soc. Sci. Fish. 38: 699-706.

Ohno, S., and N.B. Atkin. 1966. Comparative DNA vales and chromosome complements of eight species of fishes. Chromosoma 18: 455-466.

Ohno, S., J. Muramoto, J. Klein and N.B. Atkin. 1969. Diploid-tetraploid relationship in Clupeoid and Salmonoid fish. In Chromosomes Today. (Ed's.) C.D. Darlington and K.R. Lewis. Oliver and Boyd Ltd., Edinburgh. pp. 139-147.

Ohno, S., U. Wolf, and N.B. Atkin. 1968. Evolution from fish to mammals by gene duplication. Hereditas 59: 169-187.

Powers, D.A., and A.B. Edmundson. 1972. Multiple hemoglobins of Catostomid fish. I. Isolation and characterization of the isohemoglobins from Catostomus clarkii. J. Biol. Chem. 247: 6686-6693.

Prest, V.K. 1970. Quaternary Geology of Canada. In Geology and Economic Minerals of Canada. Geol. Surv. Can. Econ. Geol. Rept. No. 1. (Ed.) R.J.W. Douglas. pp. 676-764.

Roed, M. Personal Communication.

Salthe, S.N. 1969. Geographic variation of the lactate dehydrogenases of Rana pipiens and Rana palustris. Biochem. Genet. 2: 271-303.

Schultz, L.P. 1941. Fishes of Glacier National Park, Montana. U.S. Dept. Inter. Conserv. Bull. 22: 42 p.

Smithies, O., G.E. Connell, and G.H. Dixon. 1962. Inheritance of haptoglobin subtypes. Amer. J. Hum. Gen. 14: 14-21.

St. Onge, D.A. Personal Communication.

St. Onge, D.A. 1972. Sequence of glacial lakes in north-central Alberta. Geol. Surv. Can. Bull. 213: 16 p.

Svärdson, G. 1952. The Coregonid Problem. IV. The significance of scales and gillrakers. Rept. Inst. Freshw. Res. Drottningholm 33: 204-232.

Taylor, R.S. 1960. Some Pleistocene lakes of northern Alberta and adjacent areas (revised). J. Alta. Soc. Petrol. Geologists 8: 167-185.

Thorne, C.J.R., L.I. Grossman, and N.O. Kaplan. 1963. Starchgel electrophoresis of malate dehydrogenase. Biochim. Biophys. Acta 73: 193-203.

Tipper, H.W. 1971. Glacial geomorphology and Pleistocene history of central British Columbia. Geol. Surv. Can. Bull. 196.

Tsuyuki, H. Personal Communication to Dr. J.W. Clayton.

Tsuyuki, H., E. Roberts, R.H. Kerr, and A.P. Ronald. 1966. Micro starch gel electrophoresis. J. Fish. Res. Bd. Can. 23 (6): 929-933.

Van Eys, J., J. Judd, J. Ford, and W.B. Womack. 1964. On the chemistry of rabbit muscle α-glycerophosphate dehydrogenase. Biochemistry 3: 1755-1763.

Walters, V. 1955. Fishes of western Arctic America and eastern Arctic Siberia. Bull. Amer. Mus. Nat. Hist. 106 (5): 255-368.

Wheeler, J.O. 1961. Whitehorse map-area, Yukon Territory, 105 D. Geol. Surv. Can. Mem. 312.

White, A., P. Handler, and E.L. Smith. 1964. Principles of Biochemistry. 3rd Ed. McGraw-Hill Book Co., New York, Toronto, London.

Whitt, G.S., W.F. Childers, and P.L. Cho. 1973. Allelic expression at enzyme loci in an intertribal hybrid sunfish. J. Heredity 64: 54-61.

Wilkins, N.P. 1968. Multiple hemoglobins of the Atlantic Salmon (Salmo salar). J. Fish. Res. Bd. Can. 25: 2651-2663.

APPENDIX 1

A Brief History of the Wisconsin Glaciation in Western Canada

Glacial Geology and History

The last one million years have seen the advance and recession of four major ice sheets in northern North America. These glaciations were about 100,000 years each in duration and were separated from one another by longer interglacial periods (Figure i). Each of the glaciations was interrupted by shorter warmer intervals called interstades, during which ice margins pulled back peripherally, but the central mass of ice remained. In Canada, these short-term intervals are recognized only in the Wisconsin glaciation. The discovery, in the Lake Erie region, of a major interstade of some 20,000 years has enabled the division of the Wisconsin glaciation into two main stages--early and late, also referred to as pre-Classical and Classical Wisconsin. The Classical Wisconsin reached its maximum about 20,000 years ago when ice reached the vicinity of the limits of the pre-Classical stage. Most of Canada became ice free by about 7,000 years ago and time since then is referred to as Recent (Prest, 1970). Maximum limits of the Wisconsin glaciations taken together are shown in Figure ii.

Preglacial Drainages

Although it is not certain how most of central and western Canada was drained during interglacial periods, there is evidence

Figure i. Geologic time scale of Pleistocene glaciations; dates for periods below Illinoian are estimates (After Prest, 1970).

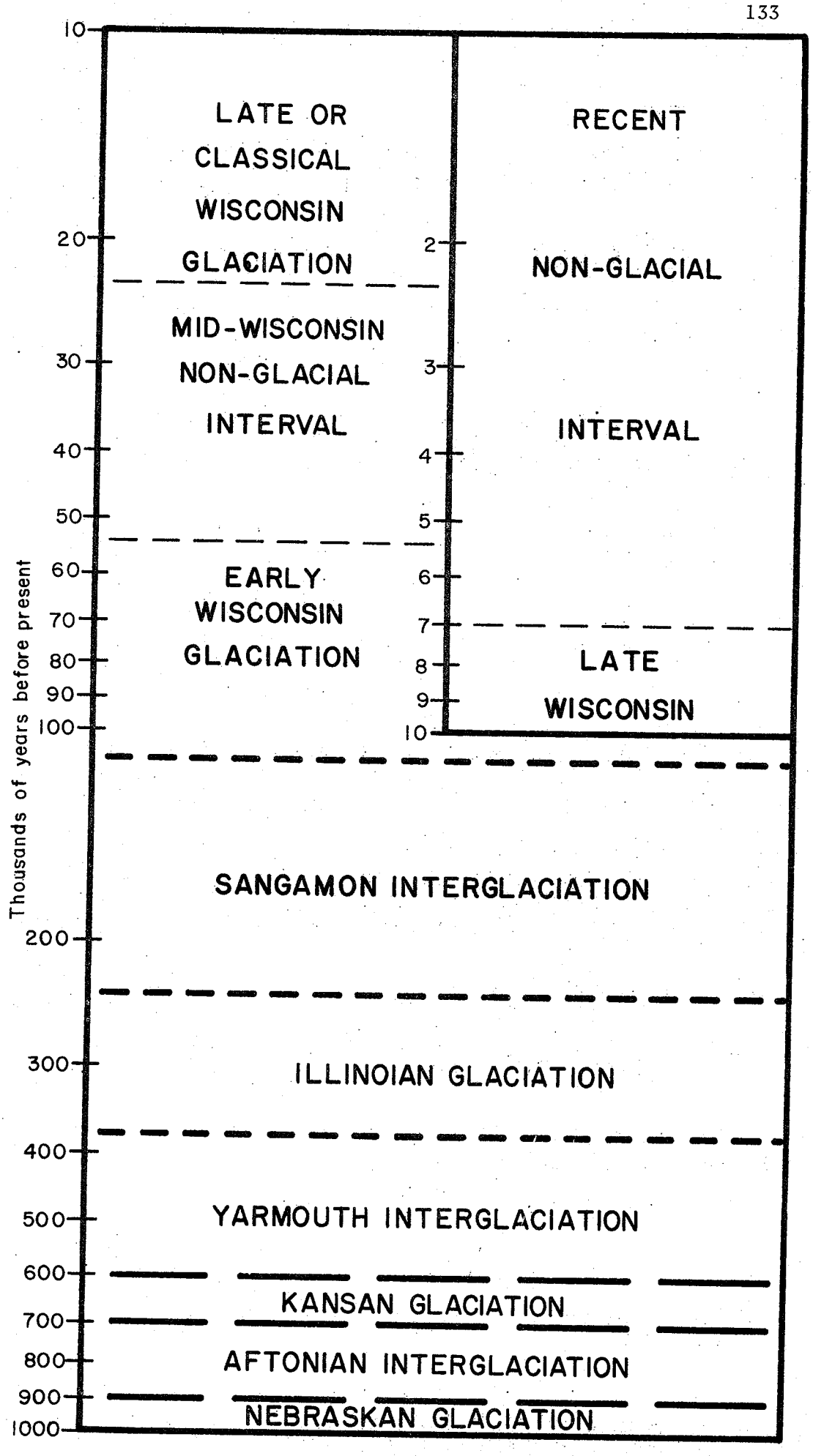
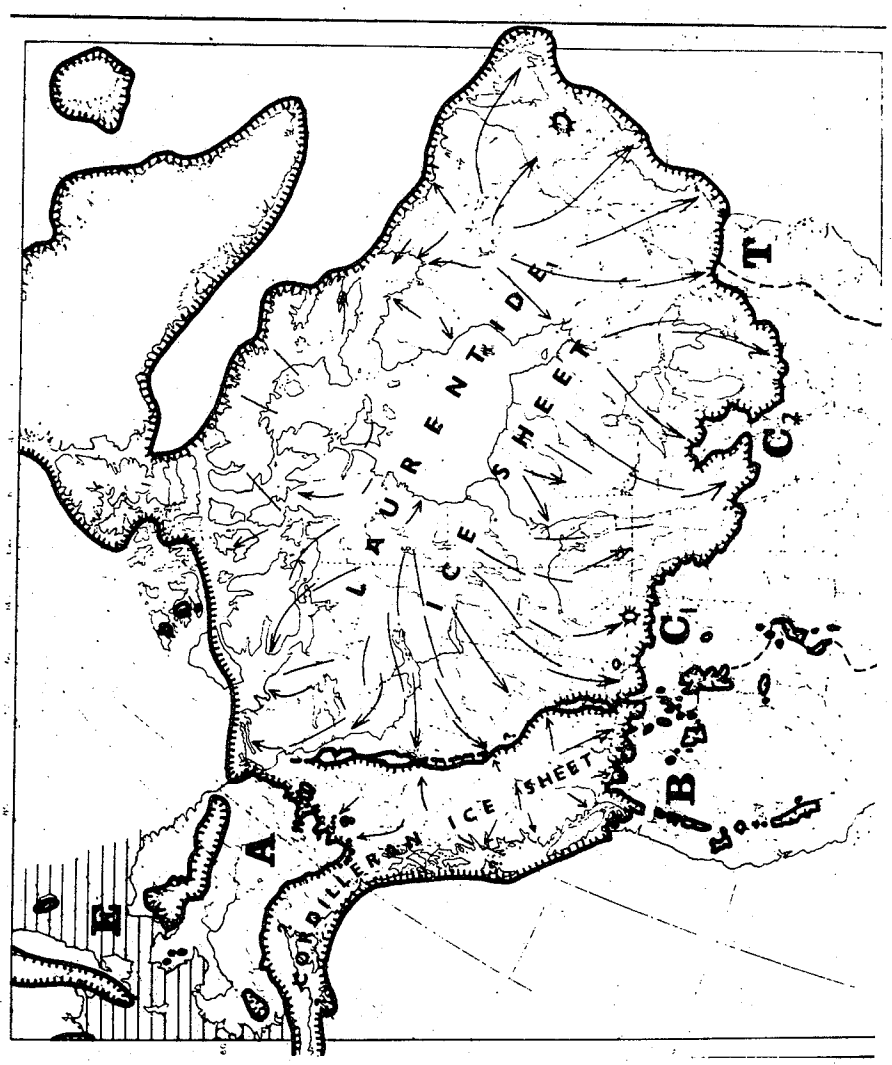

Thousands of years before present
10
20
30
40
50
60
70
80
90
100
200
300
400
500
600
700
800
900
1000
LATE OR CLASSICAL WISCONSIN GLACIATION
MID-WISCONSIN NON-GLACIAL INTERVAL
EARLY WISCONSIN GLACIATION
RECENT
NON-GLACIAL INTERVAL
LATE WISCONSIN
2
3
4
5
6
7
8
9
10
SANGAMON INTERGLACIATION
ILLINOIAN GLACIATION
YARMOUTH INTERGLACIATION
KANSAN GLACIATION
AFTONIAN INTERGLACIATION
NEBRASKAN GLACIATION

Figure ii. Maximum extent of glaciation during the Wisconsin period. Arrows suggest directions of ice advance. Letters indicate refugia for freshwater fishes: A = Alaska (Bering), B = Pacific, C_1 = Missouri River, C_2 = Mississippi River, E = Exposed Bering land bridge, T = Atlantic (From McPhail and Lindsey, 1970).


LAURENTIDE ICE SHEET
CORDILLERAN ICE SHEET
T
C2
C1
B
A
H

that pre-glacial drainages differed in some aspects from those of recent time. For example, the preglacial Milk River of Southern Alberta was a tributary of the South Saskatchewan River rather than of the Missouri River as it is at present (Prest, 1970). The Missouri River, from evidence such as northeast flowing tributaries and buried valleys, preglacially flowed northeastward to the Hudson Bay drainage but was apparently twice diverted into the Mississippi system by advancing ice sheets. The Missouri River valley was so deeply cut during the last glaciation, that the river has permanently established a southeastern drainage even though it flows at right angles to the slope of the land through which it passes. It is thought that this river course had its origins as a large ice marginal stream, thus indicating a long standing glacial boundary. In support of this, a trench roughly 150 miles long running parallel to but southwest of the present Missouri River apparently represents the abandoned channel of an older ice marginal stream (Flint, 1971). Further south, a preglacial "Great Plains Stream" draining the northwestern U.S. east of the Rocky Mountains, flowed from north to south across Kansas. To the east, the preglacial Mississippi, mainly in its present course, received a large eastern tributary, the Teays River, which drained the area around the southern margins of the Great Lakes (Metcalfe, 1966). In the northwestern plains there are indications that minor exchanges of headwaters have occurred between the Athabasca and North Saskatchewan rivers and between the Hay and Liard Rivers (Prest, 1970). Whether or not preglacial

drainages were re-established during interglacial periods is uncertain; it is equally uncertain whether or not the drainages of interglacial periods were similar to those of the present.

Glaciation

In Canada, detailed information concerning events associated with the glaciation and deglaciation of the earlier glacial periods, as well as the pre Classical Wisconsin is unavailable or obscure. The Classical or late Wisconsin events are somewhat better documented and form the basis of further discussion here.

The Wisconsin ice sheets of central and western Canada had their sources in two widely separated areas. The largest, the Laurentide Ice Sheet, is thought to have arisen in a Keewatin Center to the north and west of Hudson Bay, and in a Labrador Center in northern Quebec and Labrador. At maximum glaciation, ice from these two centers coalesced in the region of Northwestern Ontario--Manitoba. The other major Wisconsin ice sheet, the Cordilleran Glacier Complex, had its origins in the western Cordillera including the Coast, St. Elias, Monashee and Cassiar mountains, and formed ice sheet proportions by the coalescence of intermontane, piedmont and valley glaciers in these areas.

Ice from the Keewatin center of the Laurentide Ice Sheet is thought to have radiated outward to the south, southeast, southwest, west and northwest to eventually abut the Rocky Mountains at its maximum western limit. To the south and southeast, Keewatin ice coalesced with ice from the Labrador center and reached its

maximum southern limit in the region of Minneapolis, Minnesota. From there, the ice margin angled irregularly northwestward to just south of the International Boundary in northern Montana. In the north and northwest, Keewatin ice met and coalesced with westerly and southwesterly moving ice from minor centers in the Eastern Arctic and eastern Arctic Islands and reached its northwestern limits just west of the mouth of Mackenzie River, Northwest Territories. At its maximum, the Laurentide ice sheet contacted or ran over land vacated by the Cordilleran ice sheet except for restricted areas west of Great Slave Lake, west of Great Bear Lake and scattered areas further south (Prest, 1970).

Movements of Cordilleran ice were more influenced by topographic features than was the Laurentide ice sheet. From the Coast and St. Elias mountains, glaciers moved into the sea on the west, but in the interior ice flow was mainly eastward along major river valleys. In central British Columbia, ice flowed east across Nechako Plain and thence north and south along the Rocky Mountain trench. Further south, ice from the Monashee Mountains together with ice from the Coast and Cascade mountains moved to the south and southeast on the Columbia Plateau to an area about ninety miles south of the 49th parallel. In the eastern part of the Cordillera, in some places local valley glaciers and in other places the Cordilleran ice sheet, advanced over the continental divide to flow down the east slope of the Rocky Mountains. Sometimes these ice masses met the Laurentide ice sheet but at other times they had already retreated before the onset of Laurentide ice. In the upper Peace River valley, a late Cordilleran advance

overrode land recently vacated by the Laurentide ice (Mathews, 1963). Little information is available concerning interaction of the two major ice sheets in the Cordilleran region north of Peace River (Prest, 1970). In the far northern Cordillera (Yukon Territory and northwestern Mackenzie District, Northwest Territoies), ice moved northward from the St. Elias, Coast and Cassiar mountains and westward from the Selwyn and Pelly mountains. Due to the low precipitation of the area, a large portion of northwestern Yukon and much of interior Alaska was not glaciated during the Wisconsin. In the Richardson and Mackenzie mountains, glaciation was principally in the form of valley glaciers of varying extent and portions of these mountains also remained unglaciated during the Wisconsin.

Glacial Refugia

At the height of Classical Wisconsin glaciation, certain areas remained unglaciated, providing refugia for flora and fauna. The majority of the United States was unglaciated providing three principal refugia--the Atlantic seabord east of the Appalachian Mountains, the Great Plains and the Pacific Coast. From the point of view of aquatic fauna the Great Plains may have been further subdivided into a Mississippi River refugium and an upper Missouri River refugium. In the north, most of central Alaska and parts of Yukon Territory were unglaciated during the Wisconsin providing an additional refugium. Also, during the glacial maximum sea levels around the world were lowered some 200-300 feet, thus

establishing the Bering land bridge between Alaska and Siberia (Flint, 1971) (Figure ii). The Bering land bridge harboured freshwater fish species as evidenced by the presence of the totally freshwater species Cottus cognatus on St. Lawrence Island, (McPhail and Lindsey, 1970) a remnant of the former land bridge. Also, the restricted distributions of some species of freshwater fishes on both sides of Bering Strait are best accounted for by the existence of the land bridge (Walters, 1955). It is likely that the Bering land bridge was submerged as early as 11,000 years ago, at a time when most of Canada was just beginning to see the recession of the ice sheets (Walters, 1955). Smaller refugia existed near the mouths of the Anderson and Horton rivers and on Banks Island in the Northwest Territories and may have held aquatic fauna during glaciation.

Deglaciation

Features of deglaciation are generally more evident and more easily dated than glacial features. Thus knowledge of deglaciation and events immediately subsequent to ice retreat is considerably more detailed than it is for glaciation, and some details concerning ice advance are inferred from patterns of ice retreat. Generally speaking, much of ice retreat was merely in the opposite direction of glacial advance.

The Laurentide Ice Sheet began receding from the Rocky Mountains in southern Alberta about 15,000 years ago and retreated

in a northeasterly direction over the following 8,000 years, after which most of the ice sheet had disappeared. In the northwestern part of the Great Central Plains, ice similarly retreated but in an easterly to southeasterly direction. Early in deglaciation, an ice-free corridor apparently opened along the east side of the Rocky Mountains, extending from southern Alberta perhaps into the Arctic, an avenue of potentially great significance in the northward dispersal of organisms from southern unglaciated areas. Deglaciation in the Cordillera, like glaciation, was more complex than on the Great Plains. In many areas ice stagnated while in other areas local topographic features strongly influenced the recession of Cordilleran glaciers; in yet other areas, ice caps remain to the present time.

For the dispersion of fishes and other aquatic organisms, the most important feature of deglaciation in all areas was the development of extensive meltwater streams and lakes along the margins of the retreating ice sheets. These features are discussed in the next section.

Glacial and Postglacial Drainage

Knowledge about glacial and postglacial lakes and drainages is variable in scope and detail. Where possible, as much detail as is practical will be presented but discussions of the larger glacial lakes (e.g. Lake McConnell will necessarily be less detailed on a local basis. Lake Agassiz, although a large glacial lake, is a special case in this regard since it has been so well-

studied over a long period of time. Thus a reasonably detailed history of this lake (extracted from Elson, 1967) is presented as an example of the type of information that can be gained and also because it illustrates the life history and dynamics of a fairly typical glacial lake.

Glacial Lake Agassiz, at one time occupying parts of South Dakota, North Dakota, Minnesota, central Manitoba, eastern Saskatchewan and Northwestern Ontario, is perhaps the most famous and well-studied of all North American glacial lakes. This lake formed against the combined margin of the Keewatin and Labrador ice sheets as they pulled back from the Red River--Mississippi River divide. Elson (1967) presented a tentative history of Lake Agassiz, including a series of maps of the lake's various levels. This history is recounted briefly here with approximate dates for the different levels.

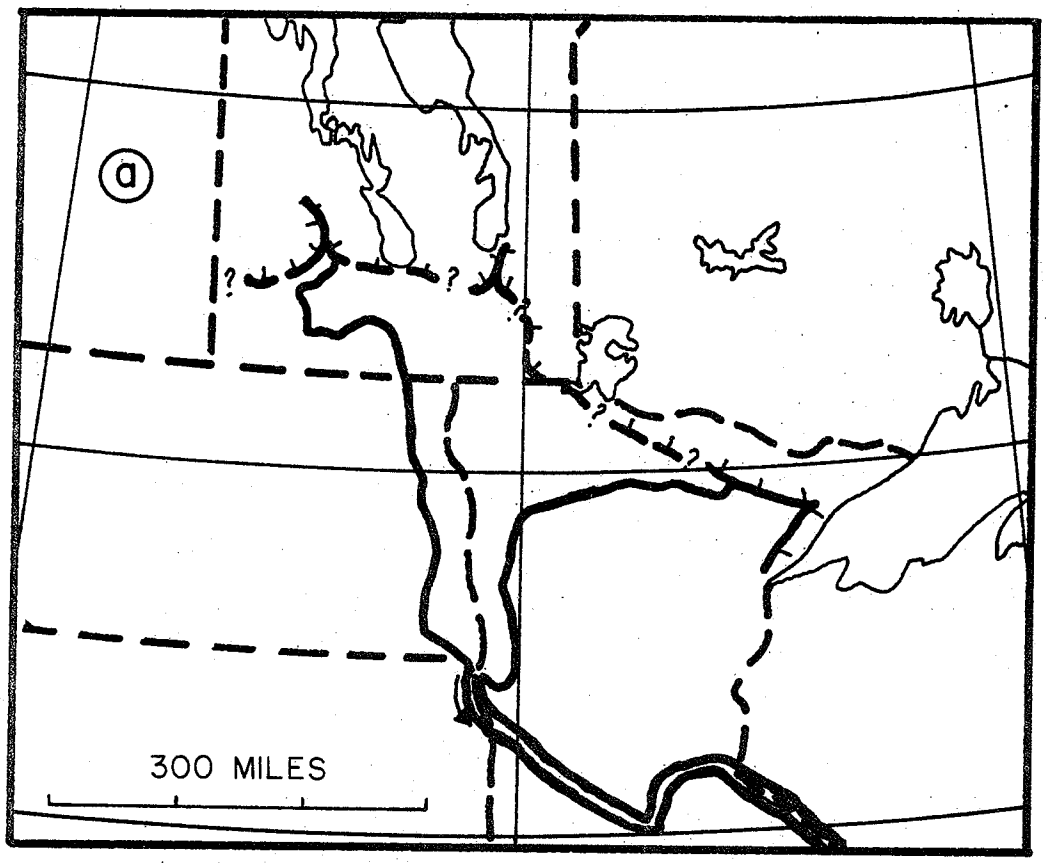
12,400--11,700 B.P. (Figure iii a) The Herman Phase

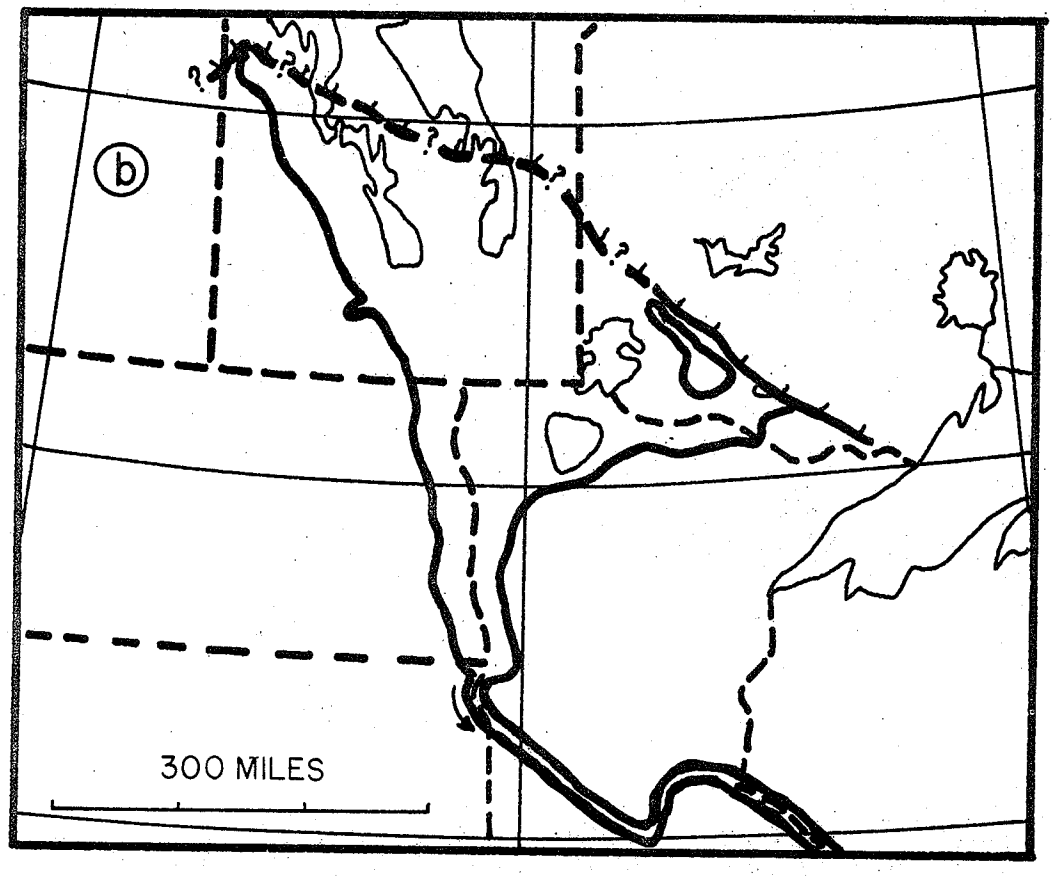
The outlet of the lake was to the south, through Lake Traverse into the Mississippi River. The Sheyenne, Elk Valley, Pembina and Assiniboine river deltas were deposited into the lake in succession. An increase in discharge, possibly due to climatic change or as a result of a shift in drainage of southern Alberta meltwaters to the South Saskatchewan River from the Big

Figure iii History of Glacial Lake Agassiz:

a) Herman phase,

b) Norcross phase.

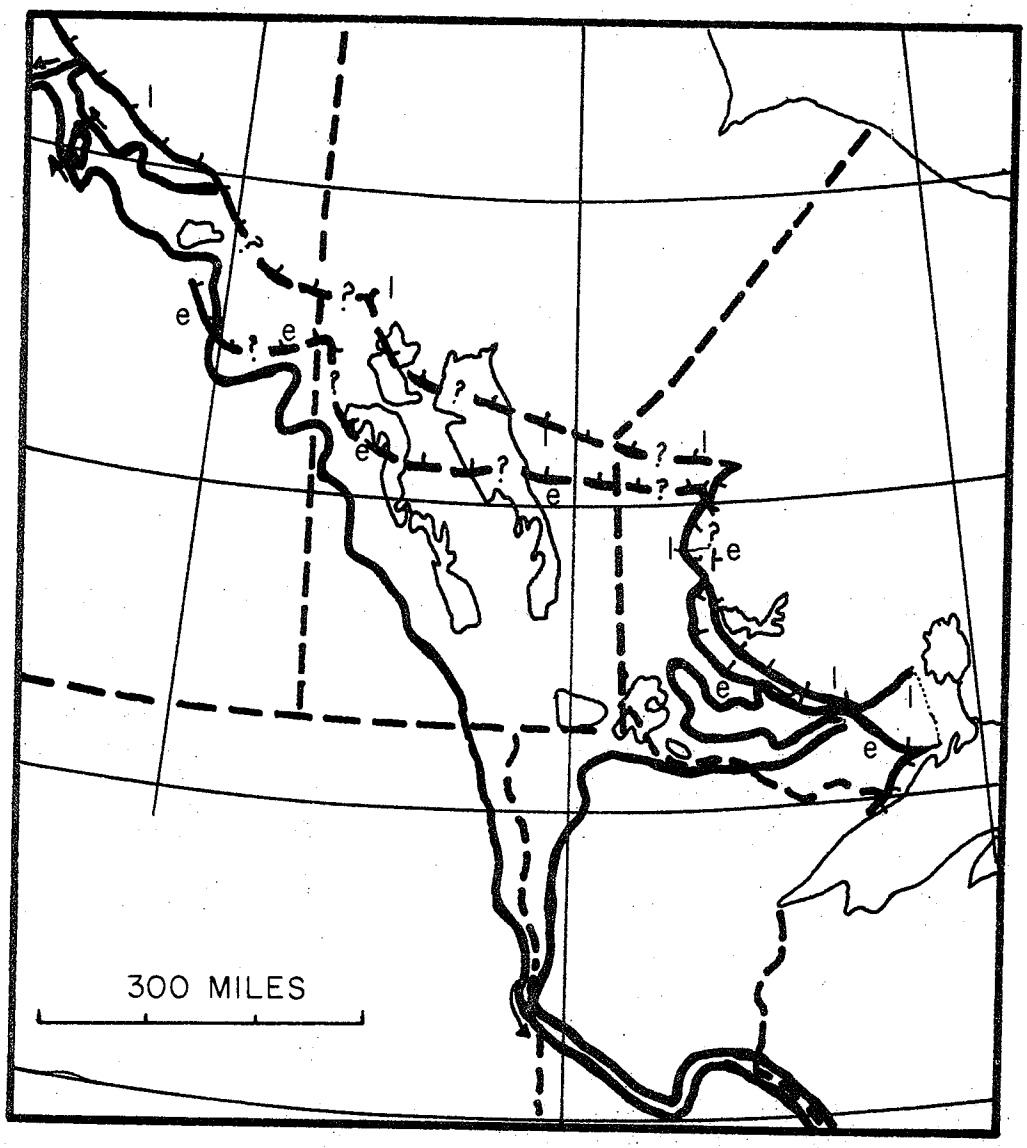

a
300 MILES


b
300 MILES

Muddy--Missouri River system is postulated, causing downcutting of the Lake Traverse outlet and lowering of the lake to the Norcross level around 12,000 B.P. (Figure (Figure iii b). The ice retreated in northern Ontario and Lake Agassiz briefly had an eastern outlet to the Lake Superior Basin via the Dog River at Coldwater Lake, resulting in the lowering of the waters to the Tintah plane. The ice readvanced to block the Dog River outlet, Lake Agassiz rose again to the Norcross level and resumed its southern outlet. Again the Lake Traverse outlet was downcut by the discharge, lowering the lake level once more to the Tintah level.

11,000--9,500 B.P. This was a period of fluctuation. There was a period of faster ice melting during which the ice margin retreated allowing the northward expansion of Lake Agassiz. The Lake Traverse outlet was further downcut to the Campbell level (Figure iv), a period during which Lake Agassiz also may have discharged to the northwest through the Flatstone Lake--Clearwater River system part of the time. The ice receded north of Lake Superior,

Figure iv. History of Glacial Lake Agassiz: Campbell phase; e = early, l = late.

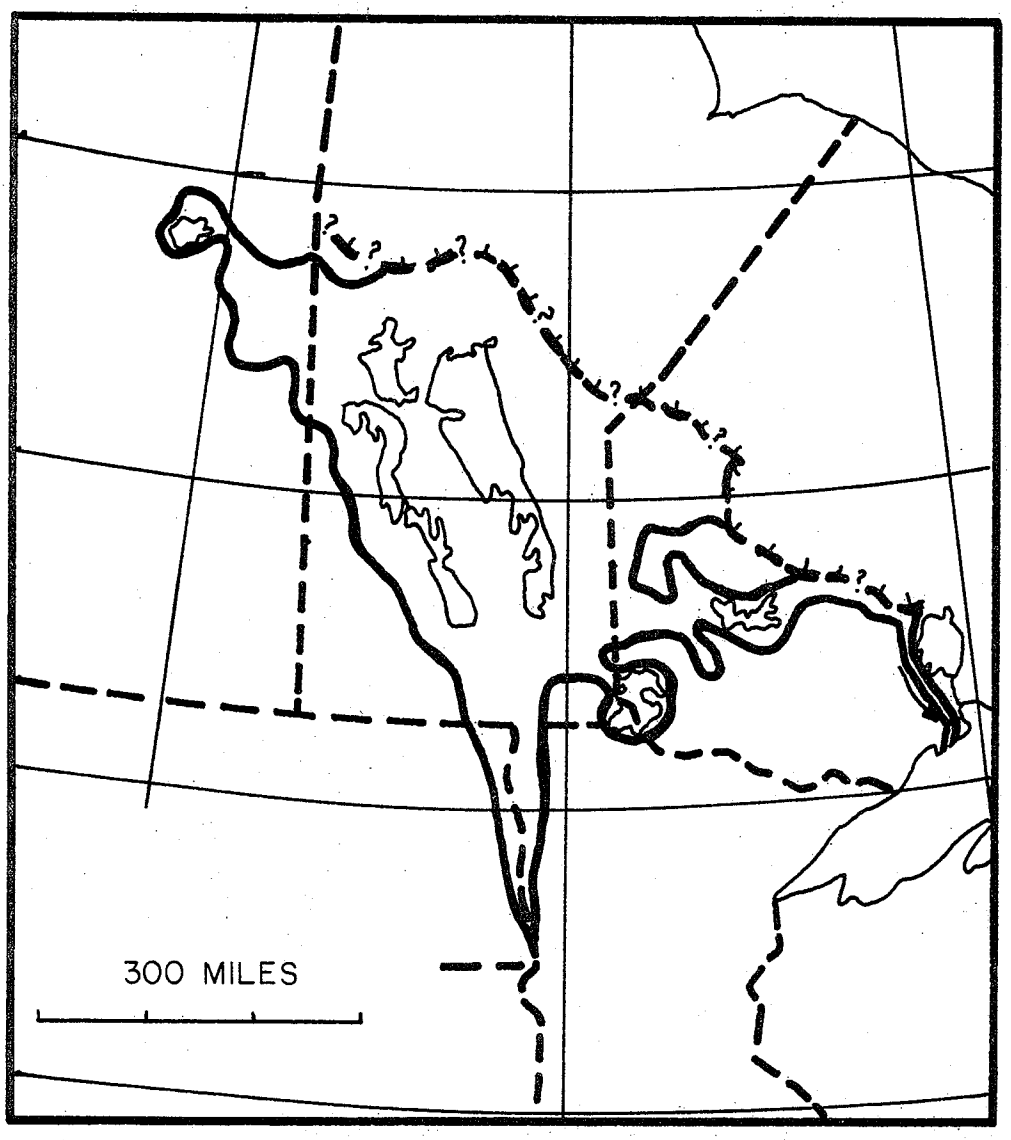

300 MILES

allowing a series of eastern outlets to to open to Lake Superior via Lake Nipigon, thus causing the lake level to fall to the Burnside level for a relatively long period.

9,000 B.P. The ice readvanced, probably due to climatic change, and blocked the more northerly eastern outlets of Lake Agassiz. The water level rose to the McCauleyville level (Figure v). Further ice advance blocked the remaining eastern outlets of the lake raising the water level for the second time to the Campbell plane (Figure iv). The lake resumed its southern outlet and may have been stable for 200-500 years. The ice margin west of Lake Nipigon retreated northward opening a series of successively lower outlets to the east causing Lake Agassiz to lower in steps to the Grand Rapids level. At the end of this period, Lake Agassiz was draining eastward through the Sandy Lake Basin, probably into Glacial Lake Barlow--Ojibway, a large glacial lake that for a time occupied a considerable portion of central northern Ontario.

8,300 B.P. A late ice advance blocked the outlet to

Figure v. History of Glacial Lake Agassiz: McCauleyville phase.


300 MILES

Glacial Lake Barlow--Ojibway, Lake Agassiz again discharged into Lake Nipigon and rose to a level somewhat lower than the Stonewall water plane (Figure vi a).

8,000 B.P. The ice margin receded and reopened the outlet to Glacial Lake Barlow--Ojibway, lowering the level of Lake Agassiz to the Gimli level (Figure vi b). Ice in Hudson Bay disintegrated, opening new lower outlets such as the Sachigo, Bigstone, Echoing and Hayes rivers.

7,500 B.P. By this time the lake had fallen to the Pipun water plane (Figure vii).

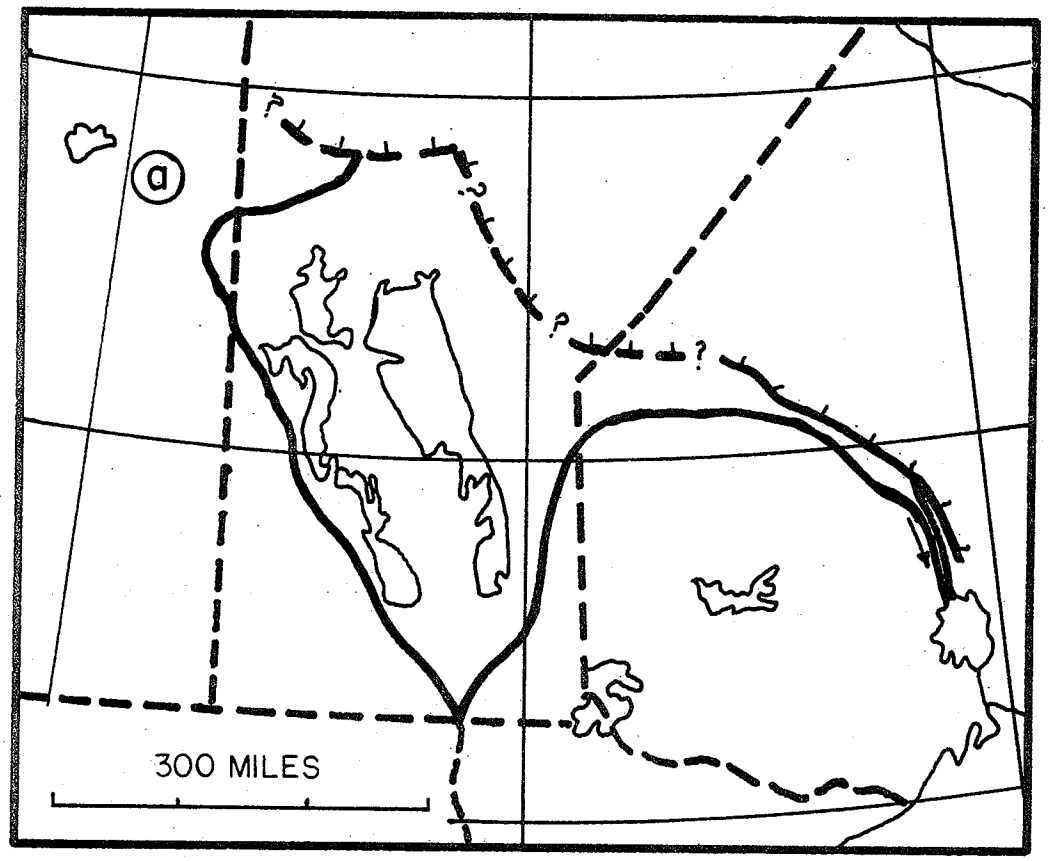
7,300 B.P. Disintegration of the last remnants of the ice sheet in the Nelson River valley allowed Lake Agassiz to drain into Hudson Bay.

Clearly, this is only a tentative history based on presently available information (Elson, 1967) but it illustrates the complexity of the history of one glacial lake. Figure viii shows the maximum limits of Lake Agassiz as a composite of all stages of the lake's history. In almost all other cases (except for St. Onge, 1972) it is only this latter type of information about glacial lakes that is available to biologists interested in the zoogeography of aquatic fauna. Clearly, inferences drawn upon such information have constraints placed upon them by the

Figure vi History of Glacial Lake Agassiz:

a) Stonewall phase,

b) Gimli phase.


a
300 MILES

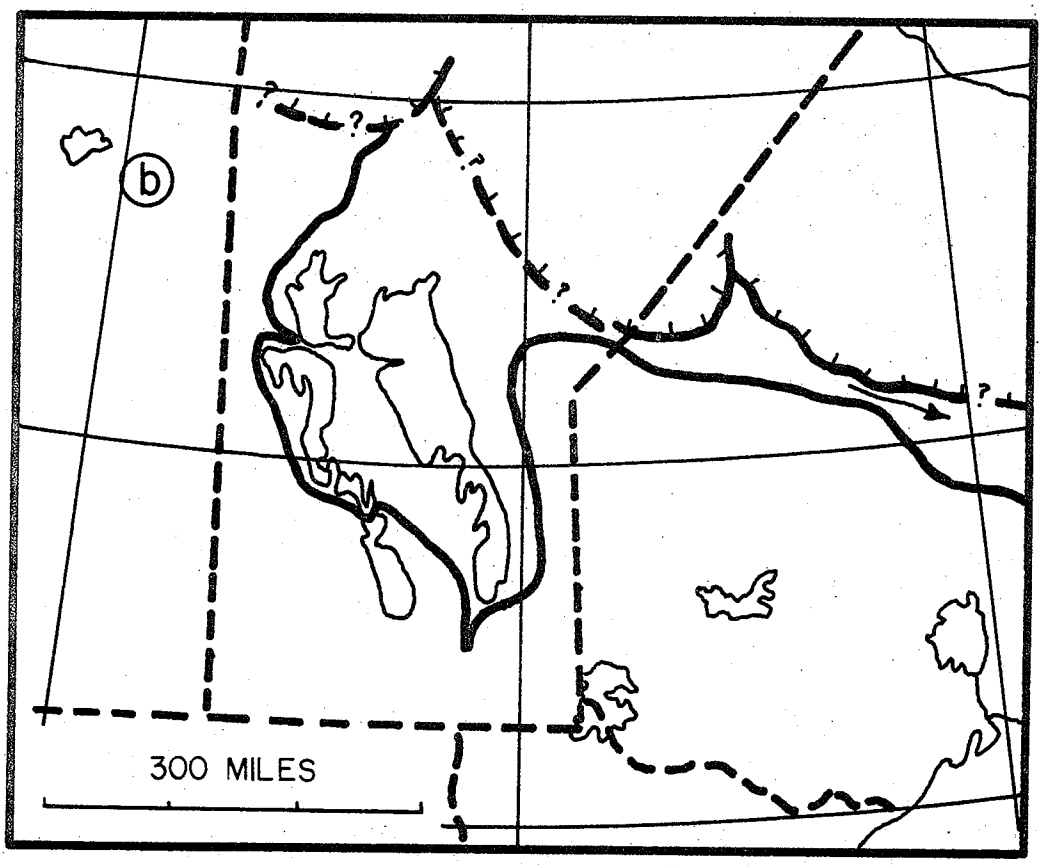

b
300 MILES

Figure vii History of Glacial Lake Agassiz: Pipun phase.

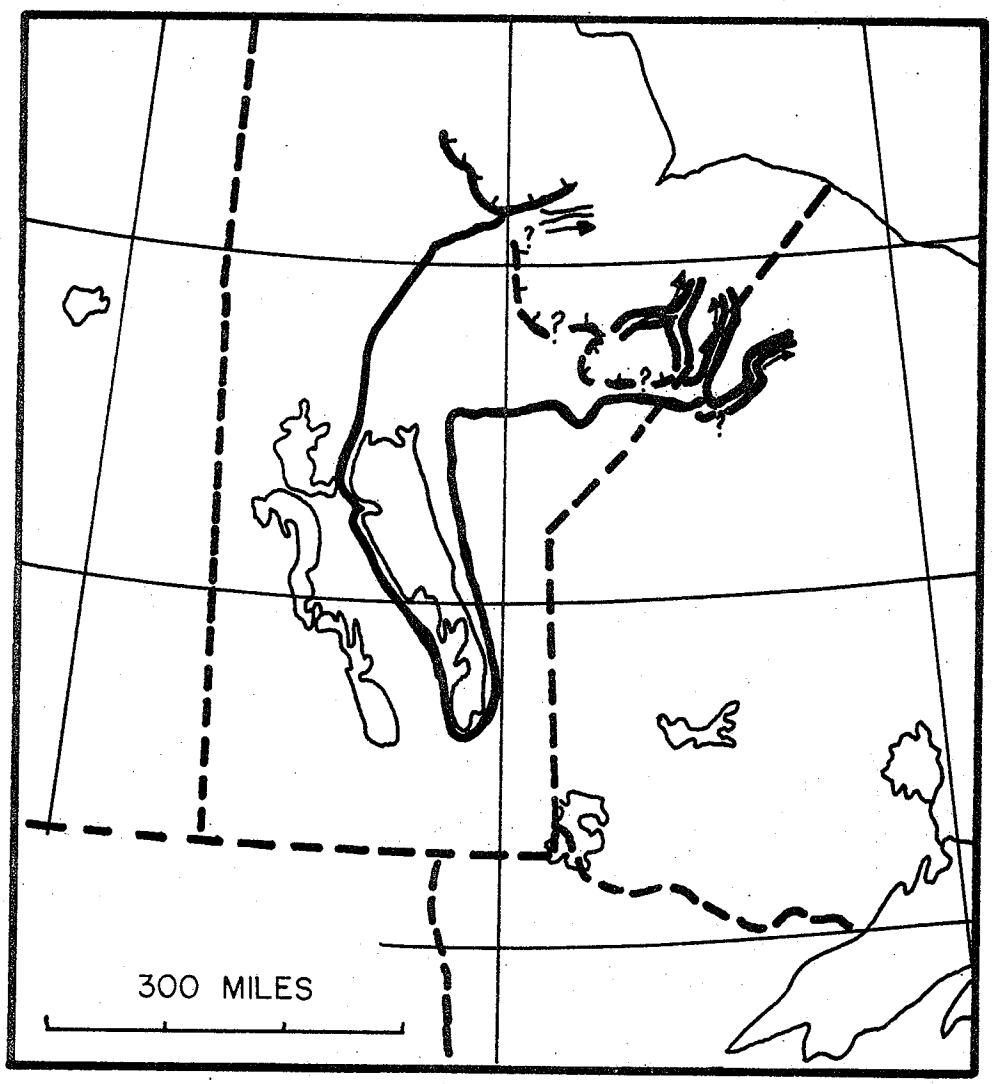
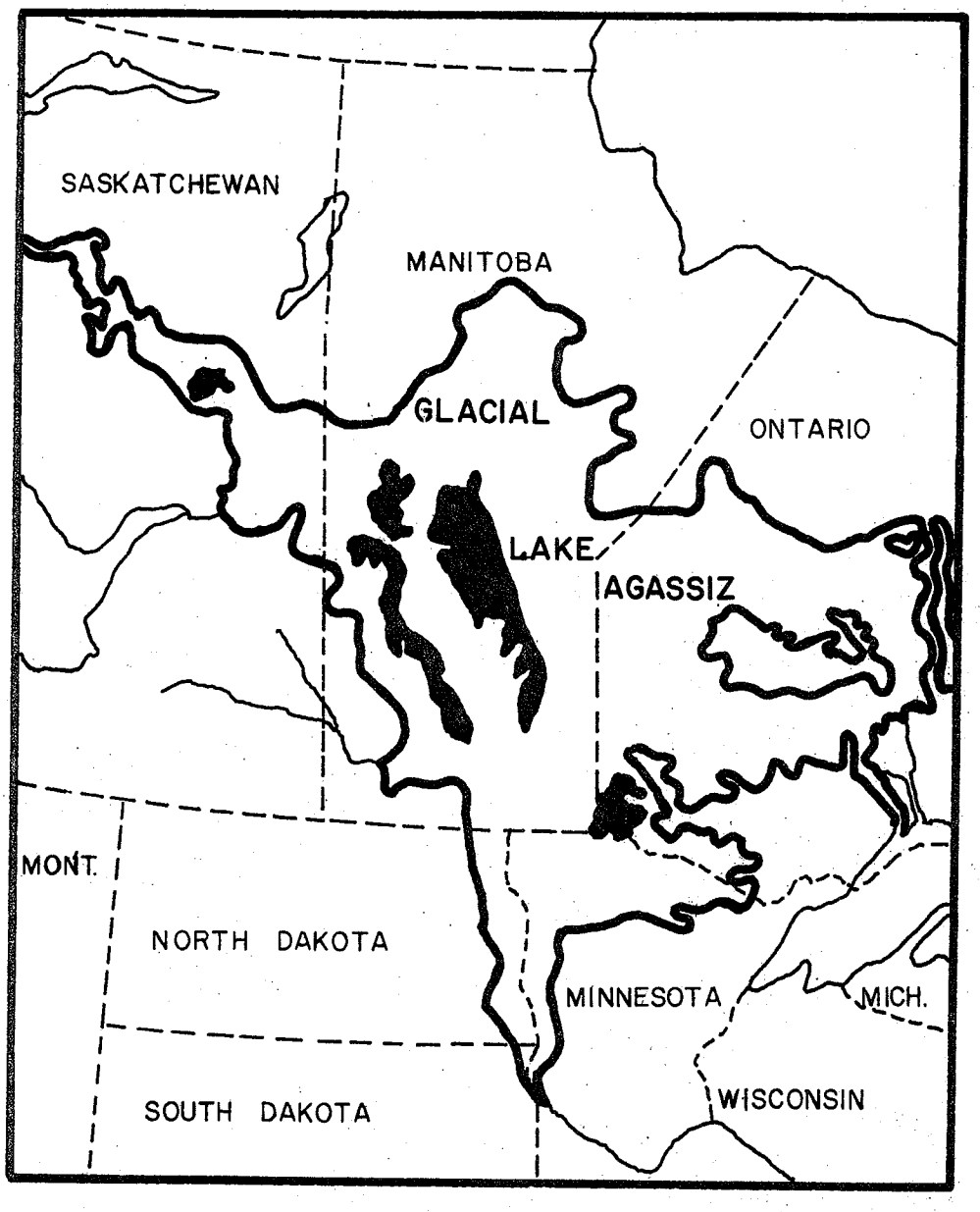

300 MILES

Figure viii Composite of Glacial Lake Agassiz phases showing maximum limits reached by the lake during its history (Figures iii to vii) from Elson, 1967).


SASKATCHEWAN
MANITOBA
GLACIAL
ONTARIO
LAKE
AGASSIZ
MONT.
NORTH DAKOTA
MINNESOTA
MICH.
SOUTH DAKOTA
WISCONSIN

quality and detail of the underlying geological observations. Most of the remaining information to follow is more general and of the less detailed type. Since the main thrust of this thesis is aimed at Western Canada, the glacial lakes east of Glacial Lake Agassiz are only very briefly considered before going on to a discussion of western glacial lakes and streams.

East of Glacial Lake Agassiz, and partly contemporaneous with it, there were two large glacial lake systems, both of which, at one time or another, apparently had connections with Lake Agassiz as well as with each other. These were Glacial Lake Barlow--Ojibway and the Glacial Great Lakes. Glacial Lake Barlow--Ojibway, in several phases during middle to late phases of Lake Agassiz (8,500-10,000 B.P.) occupied much of northern Ontario, from just north of present Lake Nipigon to north of the present Ottawa River valley. The Glacial Great Lakes also went through a complex history. In their early stages they drained to the south; the western lakes to the Mississippi River systems and the eastern lakes to the Mohawk--Hudson River system. Later, the present St. Lawrence drainage was established, although a southern outlet from Lake Michigan to the Mississippi River persisted until relatively recent time (Prest, 1970).

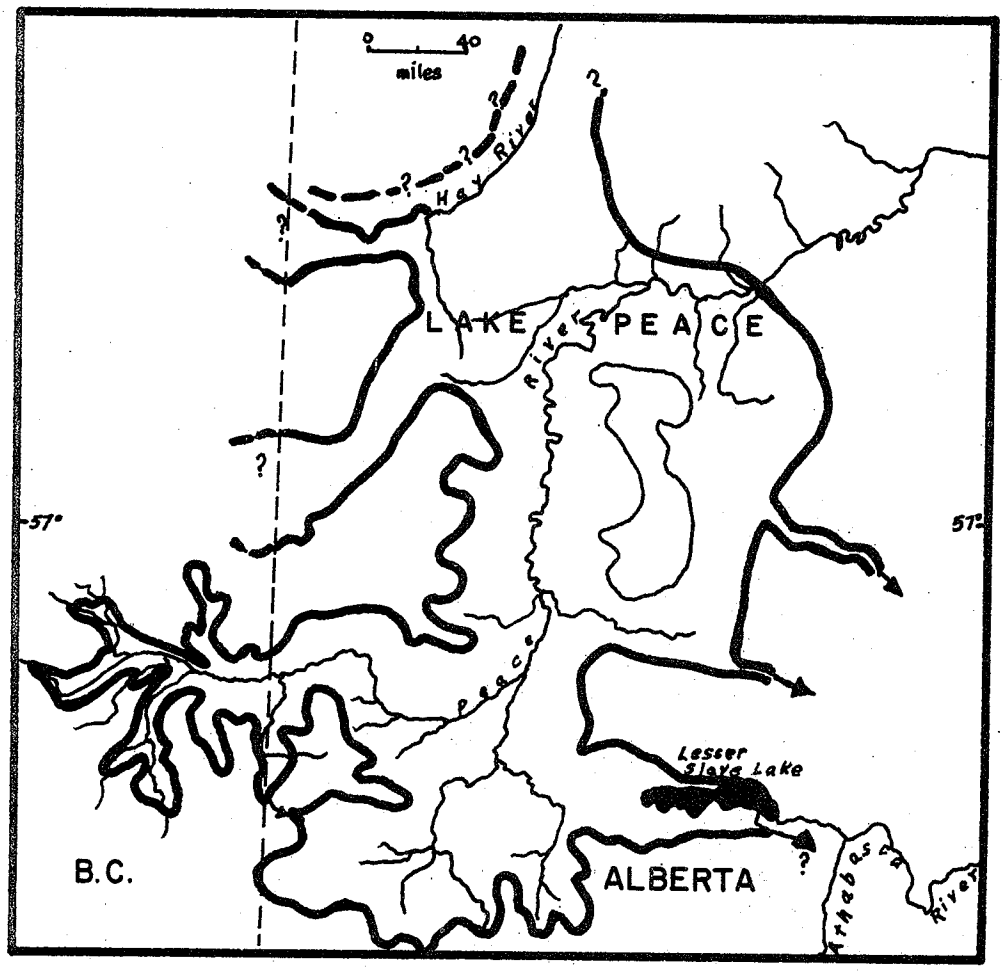
West of Lake Agassiz, along the ice front in the upper part of Qu'appelle River valley, Glacial Lake Regina developed. In early stages this lake drained via the Souris River system into the Mississippi River, but as the ice receded further to the north the lake dissipated and the valley became a spillway for lakes

in the Saskatchewan River valleys draining eastward into Lake Agassiz. As the ice receded still further northward, the upper Assiniboine valley took over this function.

In southern Alberta, small ephemeral glacial lakes developed, first discharging into the Missouri River system but as the ice receded northeastward they began draining via the South Saskatchewan River northeastward, eventually to flow into Lake Agassiz.

To the north, in central Alberta, St. Onge (1972) has described a complex series of lakes previously known as a single extensive Lake Edmonton (Taylor, 1960). St. Onge portrays a series of southeastward draining lakes of successively lower outlets along the Laurentide ice margin beginning in the north near Edson with Glacial Lake Edson around 13,500-14,000 years ago, to the final stages around 10,500 years ago with the demise of the lake series just northeast of Edmonton. The earlier lakes in the series were ponded to the west and southwest of Edmonton, but as the ice receded lakes formed in the Edmonton area, following the ice margins down the North Saskatchewan and Athabasca River valleys. Further north, the Iosegun--Fahler series of glacial lakes (St. Onge, 1972) formed the southern margins of a Glacial Lake Peace (Taylor, 1960) which was apparently contiguous in its early stages with lakes in the Edmonton area (Figure ix). Presumably all of these lakes initially were draining southeastward, eventually into Lake Agassiz, thus forming an aquatic link between the Mississippi-Great Lakes area and northwestern Great Plains. In

Figure ix. Probable limits of Glacial Lake Peace as figures by Taylor (1960).


0 40
miles
Hay River
LAKE
PEACE
River
Peace
Lesser
Slave Lake
B.C.
ALBERTA
Athabasca
River
57°
57°

fact, it is possible that the aquatic connection may have reached (via Lake Peace which extended into northeastern British Columbia) headwaters of the upper Fraser River, then draining to the north due to ice blockage in its southern reaches. Meltwater from the Coast Mountains may have at one time flowed all the way to the Atlantic in the east (Prest, 1970) and the Gulf of Mexico in the south (via the southern outlet of Lake Agassiz).

One early glacial lake in west central Alberta is a topic of controversy among geologists. That is Glacial Lake Miette, originally named by Kindle (1929) and subsequently enlarged upon by Taylor (1960). The lake was thought by Taylor (1960) to have existed principally in the upper Athabasca River valley in the Jasper area but extending up the Miette River valley and across Yellowhead Pass to near Moose Lake. Thus the divide between the upper Fraser River and the upper Athabasca River would have been bridged, connecting West Coast drainages to those east of the Rocky Mountains. However St. Onge (Personal Communication) and Roed (Personal Communication), who recently have studied the surficial geology east of Jasper, cast doubt on the existence of a Lake Miette as such, as least in Yellowhead Pass. However, these authors concede that there may have been a water connection across the divide, not necessarily in the form of a lake. The area requires further study.

West of the Rocky Mountains, in British Columbia, a large glacial lake was ponded in the Fraser River valley in the region of Prince George. This lake, known as Glacial Lake Prince George,

resulted from ice dams in the middle reaches of the Fraser River and drained to the north via Summit Lake and the Parsnip River into the Peace River system. Another glacial lake occupied Vanderhoof Basin west of Prince George and drained into Lake Prince George (Figure x). The northward drainage of the upper Fraser River persisted until the lower Fraser became ice free (Tipper, 1971). These lakes are not carbon fourteen dated at present, but it is thought (Prest, 1970) that they were contemporaneous with lakes in western central Alberta, e.g. Lake Peace.

Northern Alberta is also believed (Taylor, 1960) to have contained an extensive lake in the Athabasca Basin which in the west became confluent with Lake Peace. This lake is known as Lake Tyrrell (Taylor, 1960) (Figure xi) and supposedly first drained to the east (eventually to Lake Agassiz) but in its later stages drained northward to the Arctic. Taylor (1960) believed it possible that at one time this lake may have drained east through a gap in the Mackenzie--Hudson Bay divide near Clearwater River. Elson (1967) has postulated a similar route for a northwestward drainage of Lake Agassiz, about 10,000 years ago. Elson suggests a duration of several decades for this outlet, therefore it is possible that early stages of Glacial Lake Tyrrell drained eastward through this outlet as believed by Taylor, but as isostatic readjustments took place and Lake Tyrrell water levels declined or were displaced northward the drainage reversed allowing Lake Agassiz to drain northwestward.

Figure x. Probable limits of Glacial Lake Prince George and a lake in the Vanderhoof Basin (From Tipper, 1971).

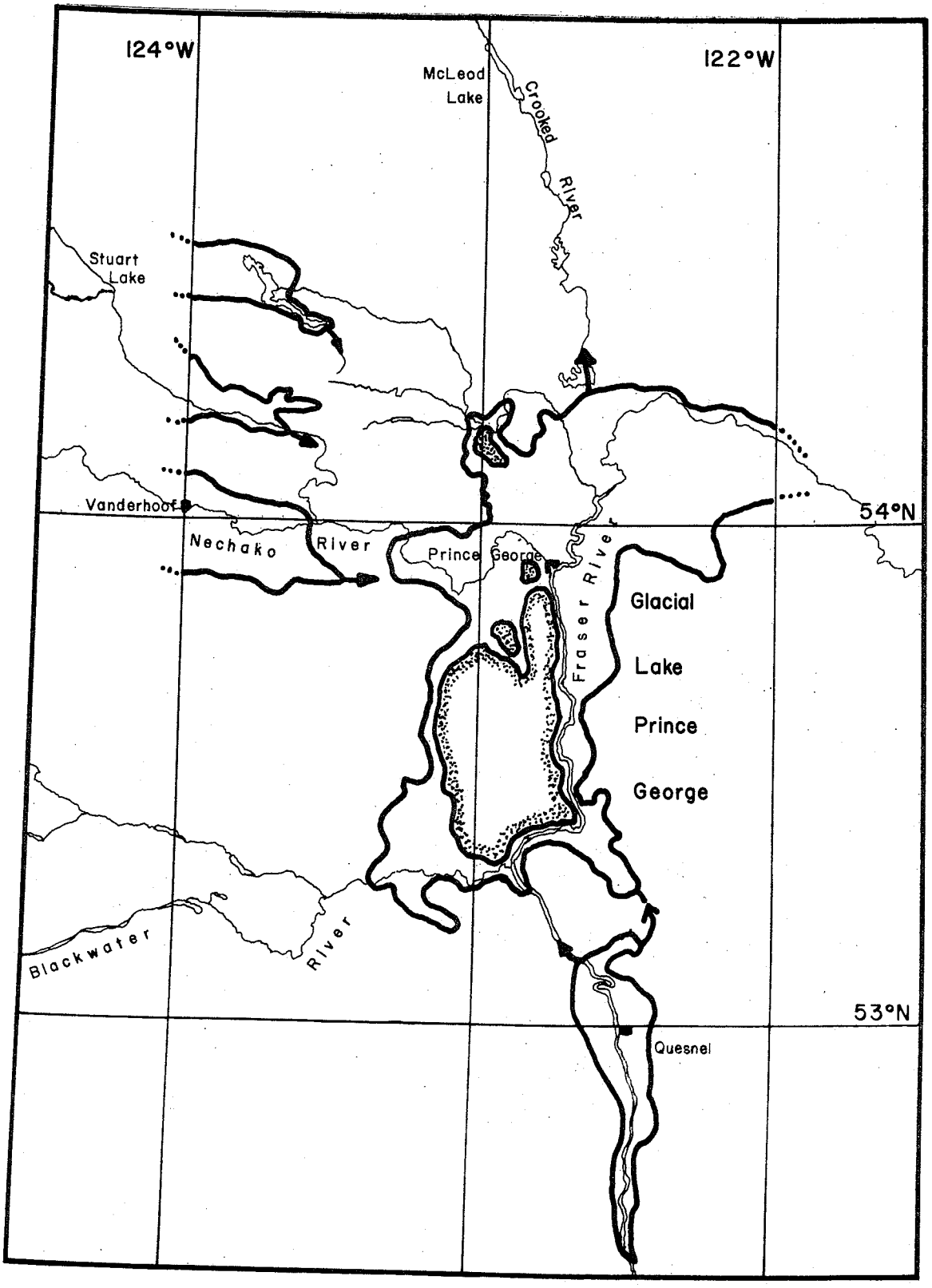
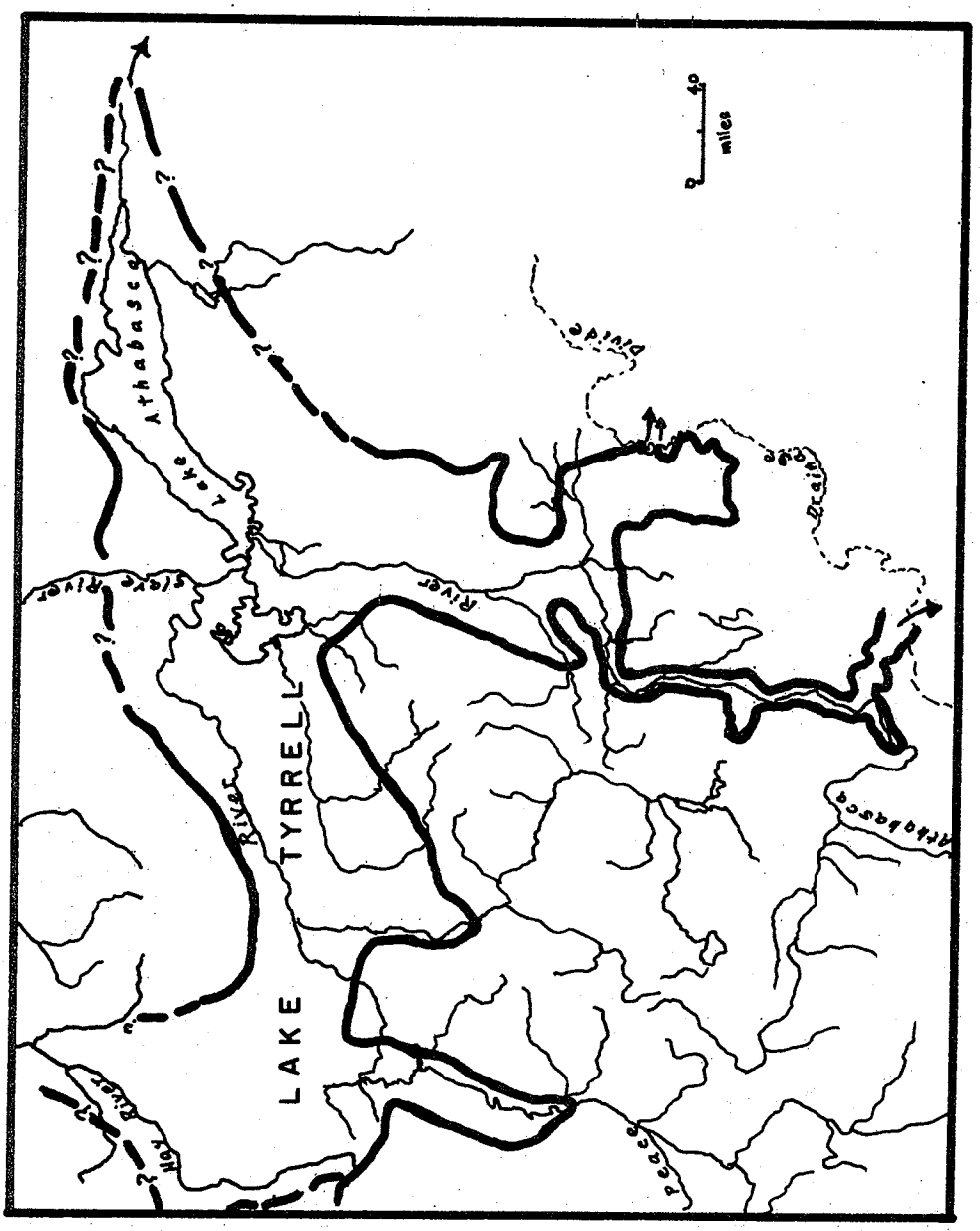

124°W
122°W
McLeod Lake
Crooked River
Stuart Lake
Vanderhoof
Nechako River
Prince George
Fraser River
54°N
Glacial
Lake
Prince
George
Blackwater River
53°N
Quesnel

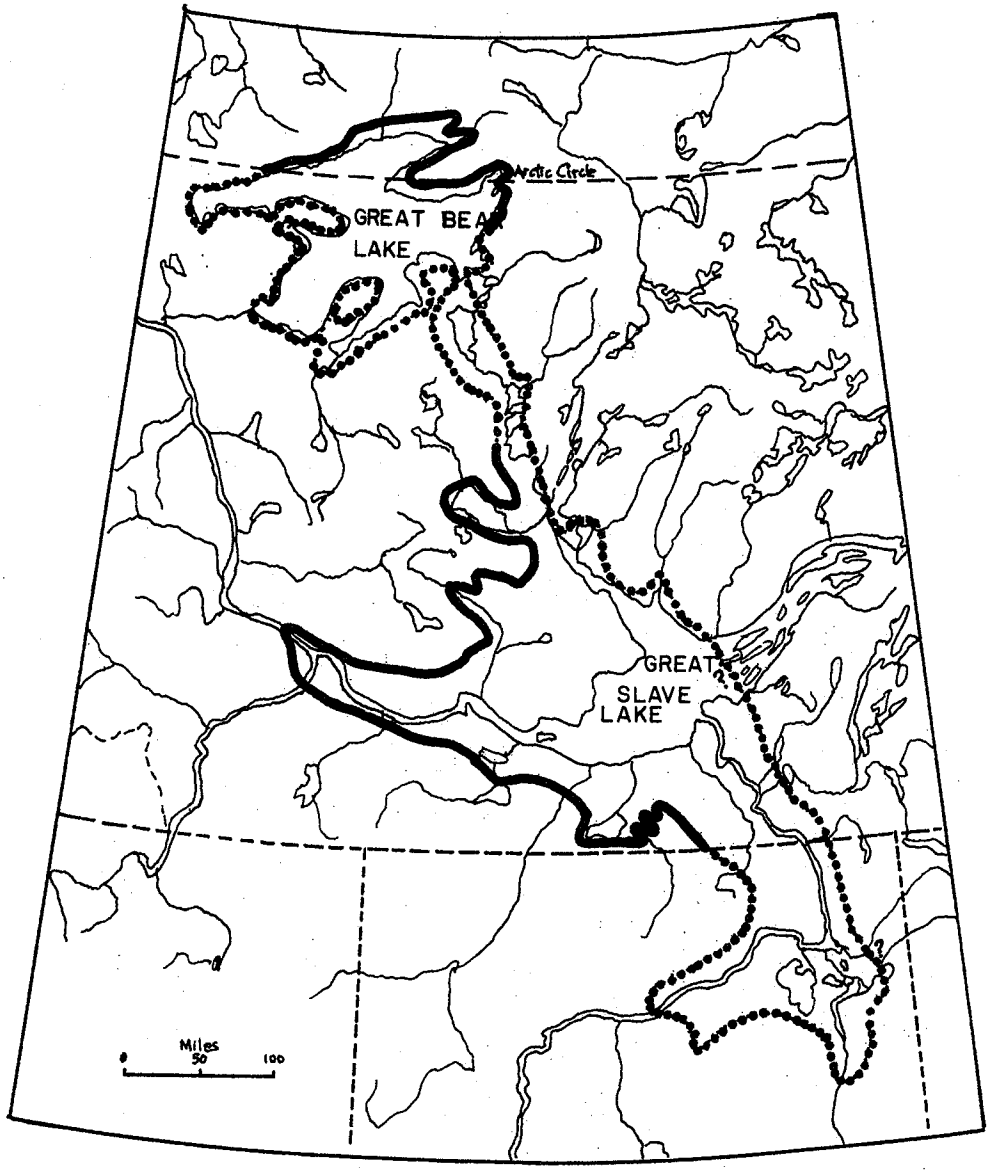
Figure xi. Probable limits of Glacial Lake Tyrrell as figured by Taylor (1960).


LAKE
TYRRELL
Athabasca
River
Peace
miles

To the north, Lake Tyrrell must have been in contact with an enormous Glacial Lake McConnell which covered, at its maximum, the basins of Great Bear, Great Slave and Athabasca lakes as well as some of the land between the basins and around each lake (Craig, 1965). Craig includes part of a lower-levelled Lake Tyrrell in his mapping of Lake McConnell (Figure xii). Craig assumed that Lake McConnell was at its maximum when the Laurentide ice margin was lying near the western edge of the Canadian Shield. This assumption would date the maximum of the lake at approximately 10,000-11,000 years ago, making it probably contemporaneous with early to middle stages of Lake Agassiz. Presumably early stages of Lake Mcconnell must have drained southeastward via Lake Tyrrell, but as ice margins receded and isostatic readjustment lifted land around the southern margins of the lake, a northerly drainage, via the Mackenzie River, was established.

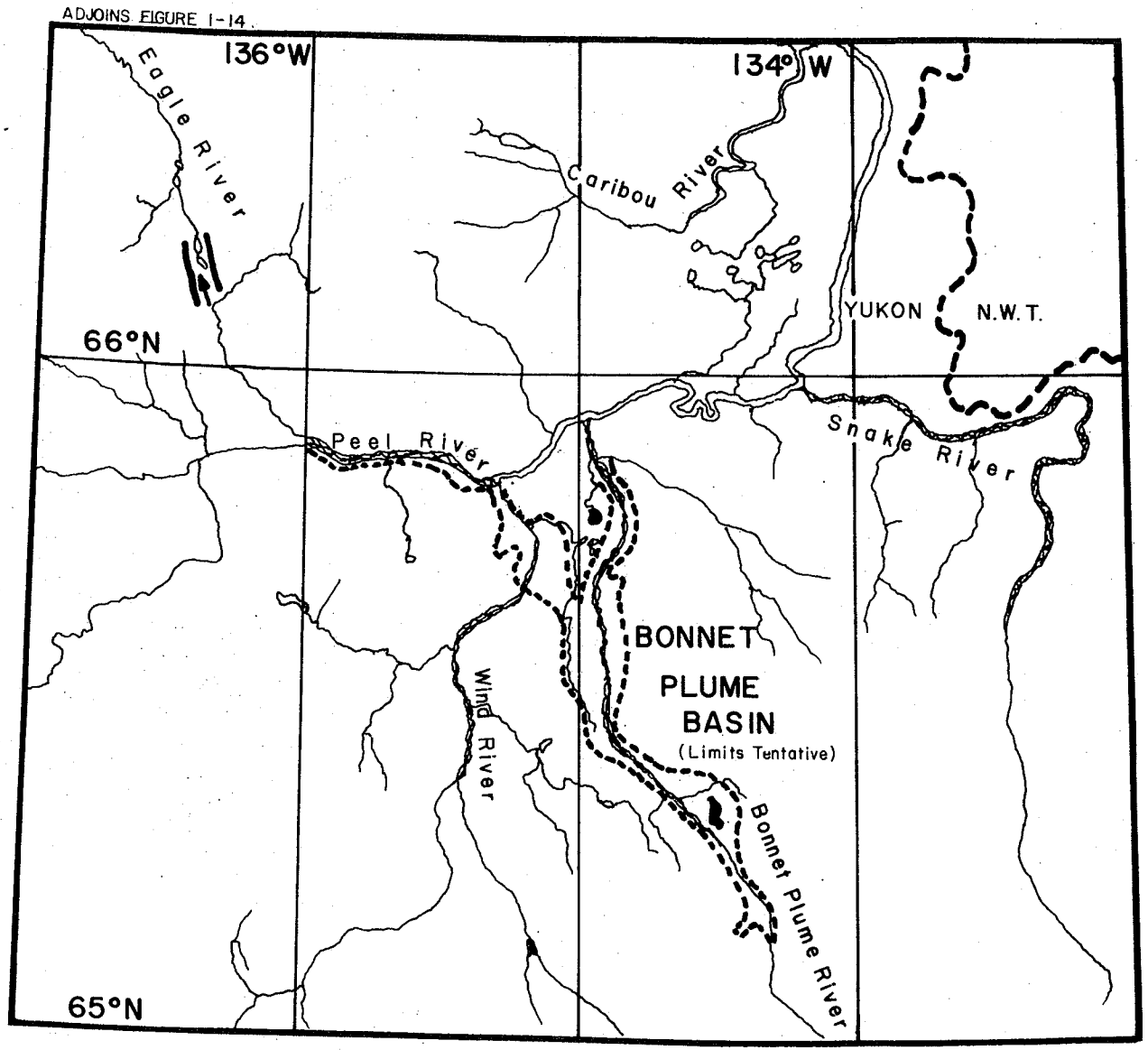
Northwest of Great Bear Lake, at earlier stages of ice retreat, Laurentide ice ponded meltwater into small ephemeral glacial lakes in nearly every valley leading out of Richardson Mountains (Hughes, Personal Communication). One area in particular, Bonnet Plume Basin, held a relatively large glacial lake which drained westward, through a pass in the mountains, into Eagle River thence to Porcupine River and finally Yukon River (Figure xiii). This lake was probably formed during both advance and retreat of both the Classical and pre-Classical Laurentide ice sheets, thus providing a significant avenue for the possible exchange of aquatic fauna between lower Mackenzie drainages (Peel,

Figure xii. Probable limits of Glacial Lake McConnell as figured by Craig (1965).



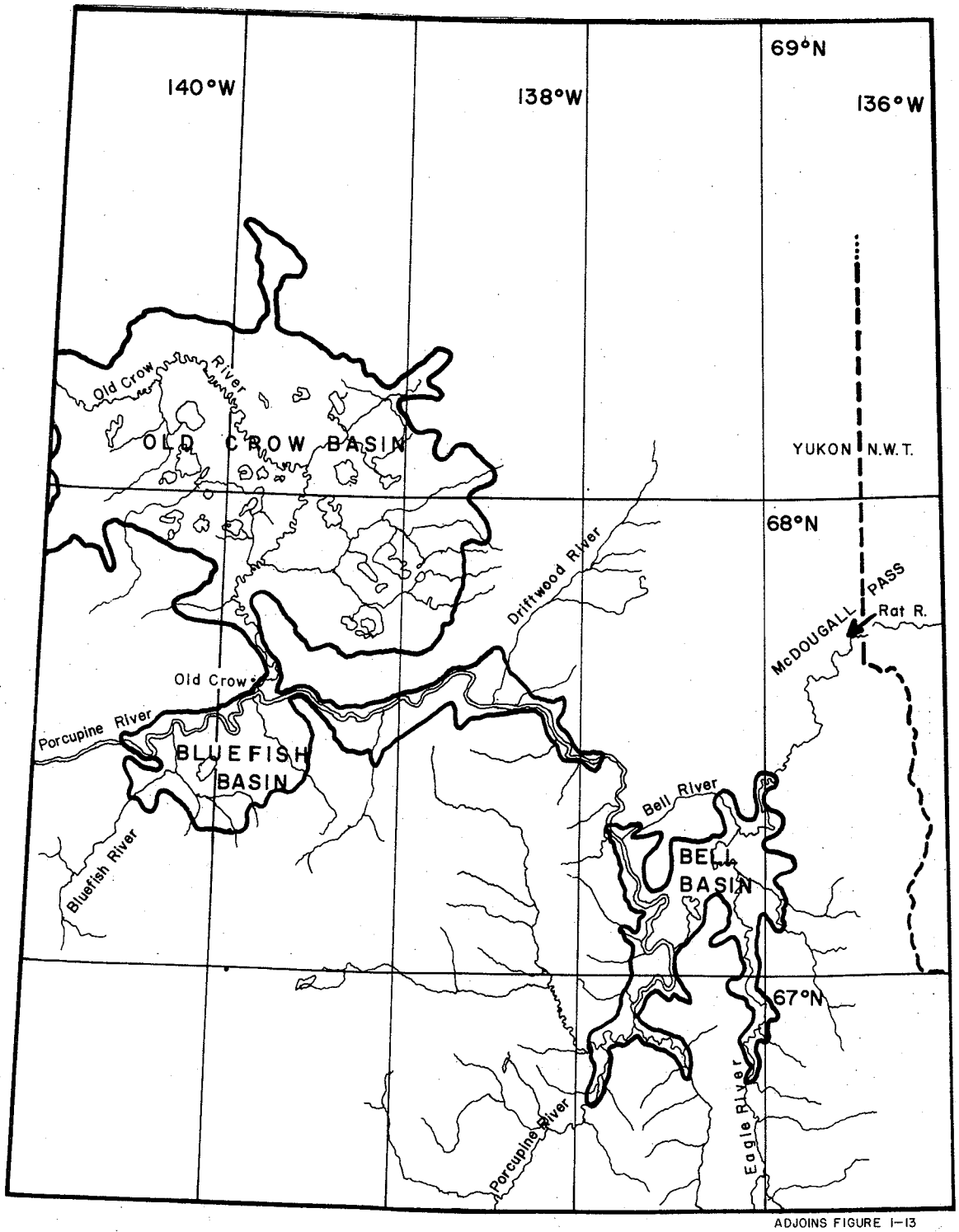
GLACIAL LAKE McCONNELL

Figure xiii. Probable limits of a glacial lake once occupying the Bonnet Plume Basin (From Hughes, 1972).


ADJOINS FIGURE 1-14
136°W
134°W
66°N
65°N
Eagle River
Caribou River
YUKON
N.W.T.
Peel River
Snake River
Wind River
BONNET
PLUME
BASIN
(Limits Tentative)
Bonnet Plume River

Bonnet Plume, Wind and Ogilvie rivers) and upper Porcupine--Yukon system drainages (Eagle River, Porcupine River) (Hughes, Personal Communication). A second major meltwater channel crossed Richardson Mountains at McDougall Pass and two smaller channels did similarly a few miles south of the pass (Hughes, 1972). All of the above watercourses emptied into the major Yukon unglaciated area which for some time was occupied to a great extent by glacial lakes in the Bell, Bluefish and Old Crow basins (Hughes, 1972, Map 1314A) (Figure xiv). The sequence of flow direction for these water courses is generally: 1) eastward drainage of the Porcupine River from east of the Ramparts, via McDougall Pass, 2) the ice advanced and blocked eastward drainage--Peel River drained a lake in Bonnet Plume Basin westward via the Palmer Lake outlet to the Porcupine River which drained westward through the Ramparts to the Yukon River +31,000 B.P., 3) the ice receded, eastward drainage through McDougall Pass was re-established plus the two smaller channels south of the pass were established, 4) late advance of Laurentide ice around 10,740 B.P. again blocked northward flow of the Peel River which again discharged westward through the Palmer Lake outlet and the Ramparts; downcutting of Ramparts, 5) McDougall Pass became ice free again but the Ramparts had been downcut to a level below that of the pass; a westward drainage of the Porcupine River was permanently established. By about 8,700-8,800 B.P., the ice had receded from the east slope of the Richardson Mountains and a northerly (Mackenzie) drainage of the

Figure xiv. Probable limits of glacial lakes once occupying the Old Crow, Bell and Bluefish basins (From Hughes, 1972).

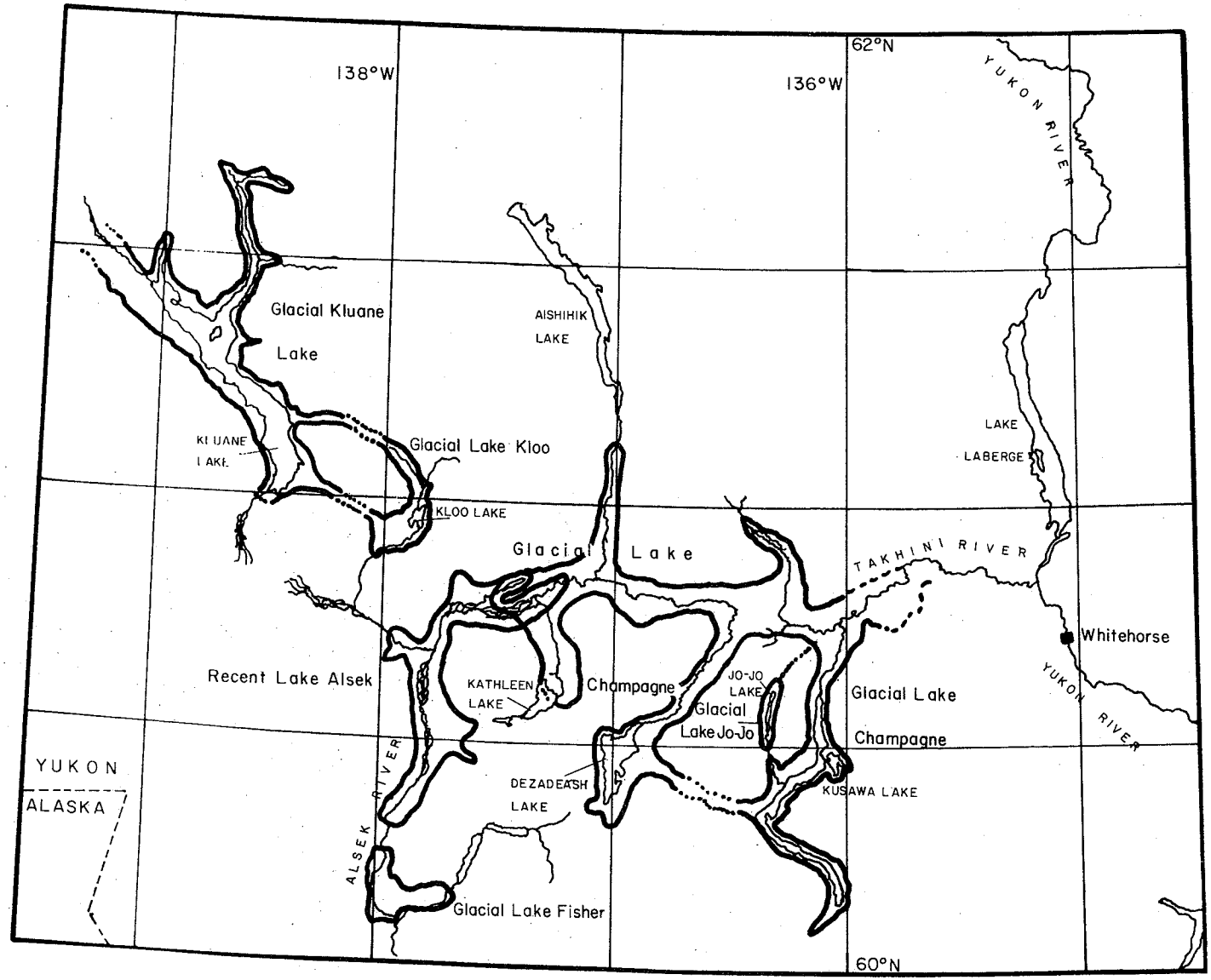

140°W
138°W
136°W
69°N
68°N
67°N
YUKON N.W.T.
Old Crow River
OLD CROW BASIN
Driftwood River
McDOUGALL PASS
Rat R.
Old Crow
Porcupine River
BLUEFISH BASIN
Bluefish River
Bell River
BELL BASIN
Porcupine River
Eagle River
ADJOINS FIGURE I-13

Peel River was established (Hughes, 1972).

To the west and south of the Yukon unglaciated area many glacial lakes formed around margins of receding ice. It is likely that glacial lakes occurred in most central parts of the valleys of the Yukon, Takhini, lower McClintock and Watson rivers (Wheeler, 1961). A large lake in central Yukon was Glacial Lake Carcross which occupied the lower parts of Wheaton River and Watson River valleys and much of Tagish and Bennett lakes. Lake Carcross was drained by the Watson River running north into the Yukon River; after the lake had drained, the Watson River assumed its present southward drainage into Bennett Lake (Wheeler, 1961). In western Yukon, an exchange of headwaters occurred between the Yukon River and the Alsek River. This exchange probably has occurred more than once--the Alsek River was ice-dammed in recent time, forming Glacial Lake Alsek about 250 years ago and also in late Wisconsin time when extensive ice in valleys of the area caused the formation of a giant Glacial Lake Champagne directly connecting the Yukon and Alsek river valleys (Kindle, 1953) (Figure xv). Kluane and Kusawa lakes were also considerably enlarged during deglaciation of this area.

This brief consideration of the Wisconsin Glaciation in Western Canada demonstrates the potentially important role that glaciation and deglaciation may have played in the distribution of aquatic fauna in northern North America. More studies such as those of Elson (1967), St. Onge (1972) and Hughes (1972) will do a great service to biological investigation into North American zoogeography.

Figure xv. Probable limits of Glacial Lake Champagne (From Kindle, 1953).


138°W
136°W
62°N
60°N
YUKON RIVER
Glacial Kluane
Lake
AISHIHIK
LAKE
KLUANE
LAKE
Glacial Lake Kloo
KLOO LAKE
LAKE
LABERGE
Glacial Lake
TAKHINI RIVER
Whitehorse
Recent Lake Alsek
KATHLEEN
LAKE
Champagne
JO-JO
LAKE
Glacial
Lake Jo-Jo
Glacial Lake
Champagne
YUKON RIVER
YUKON
ALASKA
ALSEK RIVER
DEZADEASH
LAKE
KUSAWA LAKE
Glacial Lake Fisher