THE UNIVERSITY OF MANITOBA

A BIOCHEMICAL GENETIC STUDY OF ZOOGEOGRAPHY OF LAKE WHITEFISH, *COREGONUS CLUPEAFORMIS*, IN WESTERN CANADA IN RELATION TO THEIR POSSIBLE SURVIVAL IN A NAHANNI GLACIAL REFUGIUM

by

CHRISTOPHER JOHN FOOTE

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ABSTRACT

Lake whitefish, Coregonus clupeaformis, populations from across western Canada were studied in reference to their isolation and subsequent dispersal from separate glacial refugia. Frequencies of alleles of the genes governing electrophoretic phenotypes of glycerol-3-phosphate dehydrogenase (G-3-PDH), heart-type lactate dehydrogenase (LDH) and isocitrate dehydrogenase (IDH) proved useful for characterizing populations. Hemoglobin electrophoretic phenotypes and modal gillraker numbers for each population were useful in discerning differences among large groups of populations.

Three biochemically distinct population groups of lake whitefish were found in western Canada and it is suggested that the most plausible hypothesis to account for the genetic integrity and geographical distribution of these groups is that they have separate origins in glacial refugia. Selection did not appear to account for the present genetic distinctions between the groups. It has been shown previously that lake whitefish probably survived the Wisconsin glaciation in both the Bering and Mississippi-Missouri glacial refugia. Recent geological evidence and the results of the present study regarding the distribution

of populations of one of the groups favour isolation and dispersal from an additional refugium in the area of the present Nahanni National Park, N.W.T. Lake whitefish, apparently derived from a Bering refuge stock, occupy habitats in the Yukon, Alsek, upper Liard and Peel River systems. Movement out of the Yukon River system appears to have been aided by temporary headwater exchanges. It is also probable that lake whitefish dispersed from the Bering refugium along the Arctic coast. Populations apparently derived from a Nahanni refugium stock now seem to occupy habitats in the Fraser, Peace, Athabasca, Tetcela and lower Liard River watersheds. The most plausible dispersal route for the ancestral populations was via waterways in an ice free corridor which probably existed along the eastern foothills of the Rocky Mountains during late Wisconsin glaciation. Most of the populations of the plains of western Canada are probably derived from a Mississippi-Missouri refugium stock. Dispersal appears to have been via connections of the large glacial lakes which occupied a large proportion of the plains during deglaciation.

Contact of the different refugial forms appears to have led to introgression in some cases but, in general, most populations remain genetically distinct even in the absence of physical barriers to gene flow.

This thesis is dedicated to my mother,

Margaret Lillian (Peggy) Bruce

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INTRODUCTION

The amino acid sequence of a given enzyme appears to evolve at approximately the same rate irrespective of the organism in which it occurs (Kimura and Ohta 1974, Sarich 1977, Wilson et al. 1977). The rates vary among different classes of proteins, depending on their functional complexity (Kimura and Ohta 1974, Sarich 1977, Wilson et al. 1977). For example, Sarich (1977) pointed out that in apparently all organisms plasma proteins and various other secreted proteins undergo amino acid substitution at a ten times greater rate than intracellular enzymes involved in complex metabolic pathways such as glycolysis. The discovery that within any given class of proteins the rate of amino acid substitution appears to be nearly solely dependent on time is proving useful in the fields of paleontology, anthropology, and systematic biology (Wilson et al. 1977). These studies are complicated by the effects of population bottlenecks whereby alleles may be lost due to a combination of founder effect and genetic drift, leading to an inaccurate estimation of the time elapsed since the separation of different populations (Chakraborty and Nei 1977). On the other hand, allele losses arising from population bottlenecks can be useful in distinguishing populations. For example, Avise and Selander

(1972) used genetic differences they concluded to be caused by genetic drift to show that there was little interbreeding between the surface and cave-dwelling fish species of the genus Astyanax. Therefore both the occurrence of new substitutions (i.e., new alleles) and the loss of alleles in separated populations can prove useful in measuring the integrity of these stocks if contact is ever re-established.

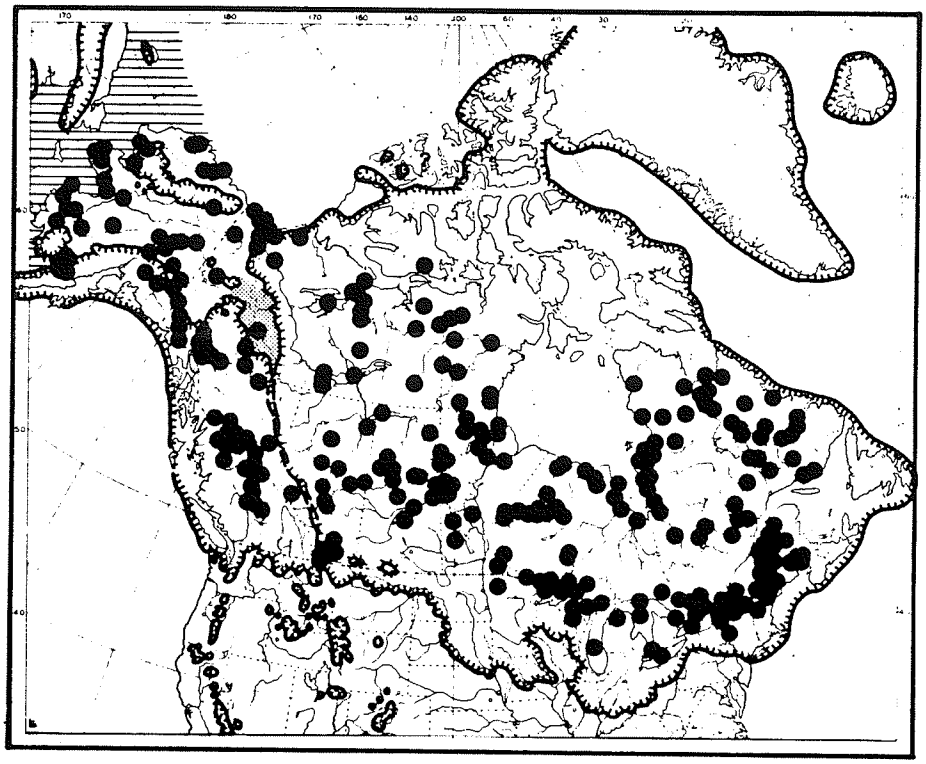
Fish populations separated by the effects of pleistocene glaciation often show biochemical divergence (Lindsey et al. 1970, Avise and Selander 1972, Avise and Smith 1974, Franzin and Clayton 1977, Bodaly and Lindsey 1977, Wiseman et al. 1978, Lynch and Vyse 1979, Ryman et al. 1979). These biochemical differences can be used to establish probable areas of population survival during glaciation and to outline the extent of dispersal and intergradation since deglaciation.

Lake whitefish, Coregonus clupeaformis, exhibit considerable and continuous morphological variability over their range in North America and it is mainly for this reason that the species is referred to as a 'complex' by McPhail and Lindsey (1970). Some of this morphological plasticity has been attributed to the isolation of populations in separate refugia during the time when the Wisconsin ice sheets covered most of Canada (Lindsey et al. 1970). These refugial populations were probably separated for at least 50,000 years.

Deglaciation allowed the dispersal of these separate stocks from their respective refugia to nearly all of Canada (Fig. 1). The lake whitefish populations of western Canada were considered by McPhail and Lindsey (1970) to have dispersed from two discrete refugia: the Bering (unglaciated parts of the Yukon and Alaska) and Mississippi-Missouri (unglaciated northern sections of the watersheds of these rivers). Recently, Ford (1976) discovered evidence for the existence of at least one glacial lake during the Wisconsin glaciation in Nahanni National Park, Northwest Territories, which may have also served as a refuge for lake whitefish populations.

The distribution of lake whitefish in western Canada, with special reference to glacial refugia, was studied biochemically by Franzin and Clayton (1977). They concluded that lake whitefish populations had dispersed from the Mississippi-Missouri and Bering refugia and mixed throughout most of western Canada. In contrast, populations in the upper Liard, Alsek and Yukon River watersheds were apparently derived solely from the Bering population. This distinction was postulated to be preserved by physical barriers such as the Liard canyon which prevented gene flow from the Mississippi-Missouri populations from reaching the Bering populations. Dispersal from the Bering refugium has probably occurred via the upper Liard River system (Franzin and Clayton 1977), the

Figure 1. Distribution of native lake whitefish populations in North America in relation to the maximum extent of Wisconsin glaciation (from Lindsey et al. 1970).



Peel River system (Bodaly and Lindsey 1977) and movement along the Arctic coast (McPhail and Lindsey 1970). One population on the headwaters of the Flat River, a tributary to the Nahanni River, was found to be genetically distinct from all other lake whitefish populations sampled (Franzin and Clayton 1977). It is now suggested that the existence of this population supports the idea that lake whitefish may also have survived Wisconsin glaciation in a Nahanni refugium.

The purpose of this study was to investigate the apparent dispersal of lake whitefish out of the Bering refugium and their subsequent primary zones of contact with other stocks of lake whitefish derived from populations which survived glaciation in other refugia.

Possible primary zones of contact are examined from across western Canada with special emphasis on the Liard River system where populations which may have survived in a Nahanni refugium would also be expected to come in contact with fish dispersing from the Bering and Mississippi-Missouri refugia.

METHODS

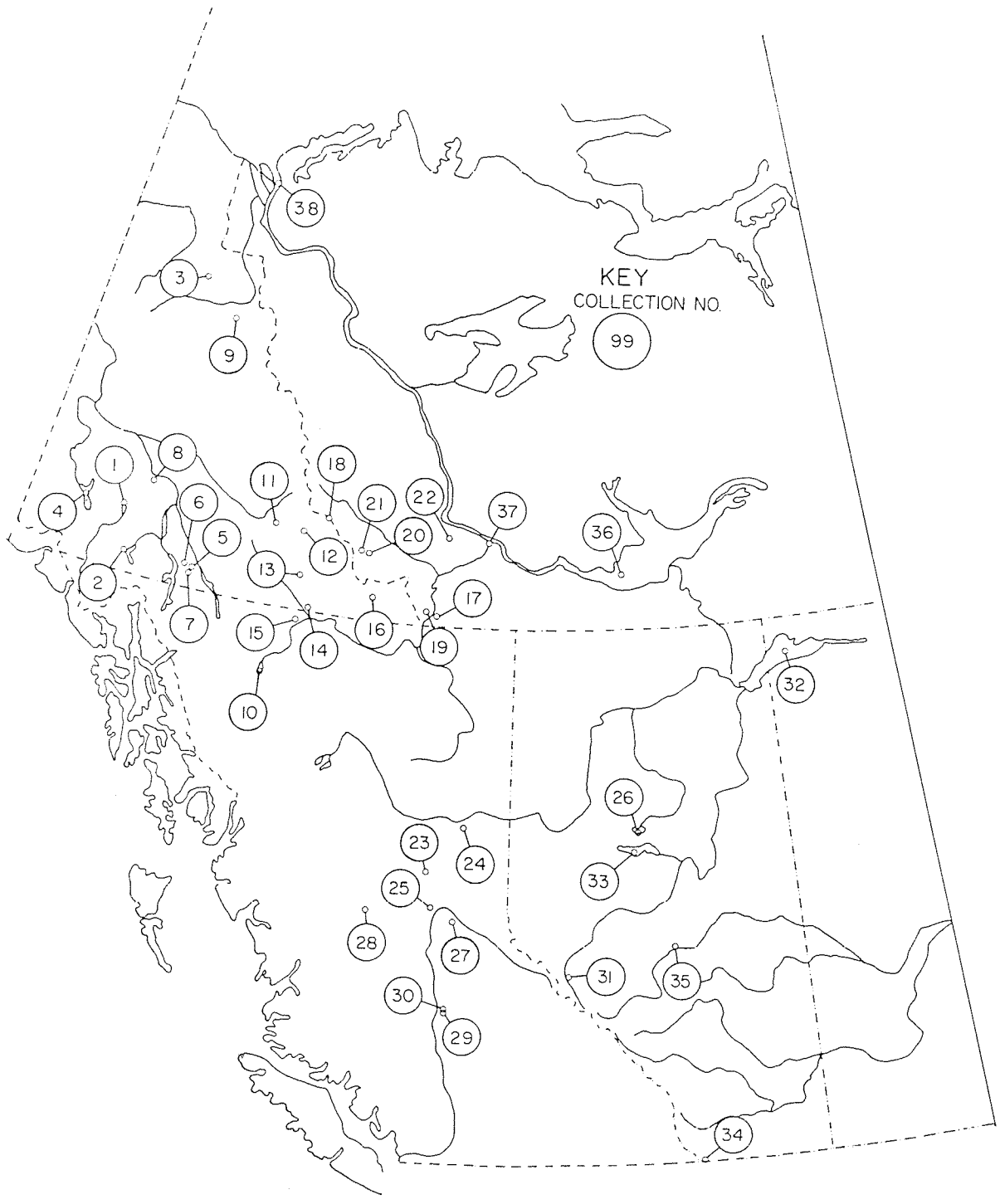
Collection of Lake Whitefish

Lake whitefish were collected from 38 locations from across western and northwestern Canada (Fig. 2) using experimental gill nets (two types: both 38.1 m long and 2.1 m deep with panels in order of either 2.54, 5.08, 7.62, 3.81 and 6.35 cm or 3.81, 7.62, 11.48, 5.08 and 10.02 cm stretched mesh monofilament nylon) over the period of 1970 to 1978. Fish carcasses were either iced or frozen directly after capture, and within four days, stored at -40C until required for further analysis. Hemoglobin samples from lake whitefish were taken, stored and shipped to our laboratory in Winnipeg following the methods outlined by Lindsey et al. (1970). Electrophoresis was performed within 7 days of collection.

Biochemical Analysis

Chemicals used in this study are abbreviated as follows: nicotinamide-adenine dinucleotide phosphate (NADP), N,N-bis-(2-hydroxyethyl)-glycine (Bicine), phenazine methosulphate (PMS) and 2;2'-di-p-nitrophenyl-5,5'-diphenyl-3-3- (3,3'-dimethoxy-4,4'-biphenylene)-ditetrazolium chloride (Nitro BT, NBT).

Figure 2. Collection sites for lake whitefish populations from western Canada. Numbers on map correspond to those of Table 2.


KEY
COLLECTION NO.
99
38
3
9
1
8
4
6
11
18
21
22
37
5
20
36
12
2
13
17
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Phenotypes for four isozyme systems were visualized following the semi-micro electrophoresis method of Tsuyuki et al. (1966a). The enzyme or protein examined, tissue sampled and exact methods of electrophoresis are given in Table 1.

The nomenclature for the genetic basis of LDH, IDH and G-3-PDH follows the system proposed by Bailey et al. (1976) for the duplicated salmonid LDH loci. LDH isozyme nomenclature (Franzin and Clayton 1977) and IDH isozyme nomenclature (Bodaly 1977) have previously been presented in this form. The G-3-PDH isozyme designations of Clayton et al. (1973) are converted to the format proposed by Bailey et al. (1976) in this study (see Results; G-3-PDH).

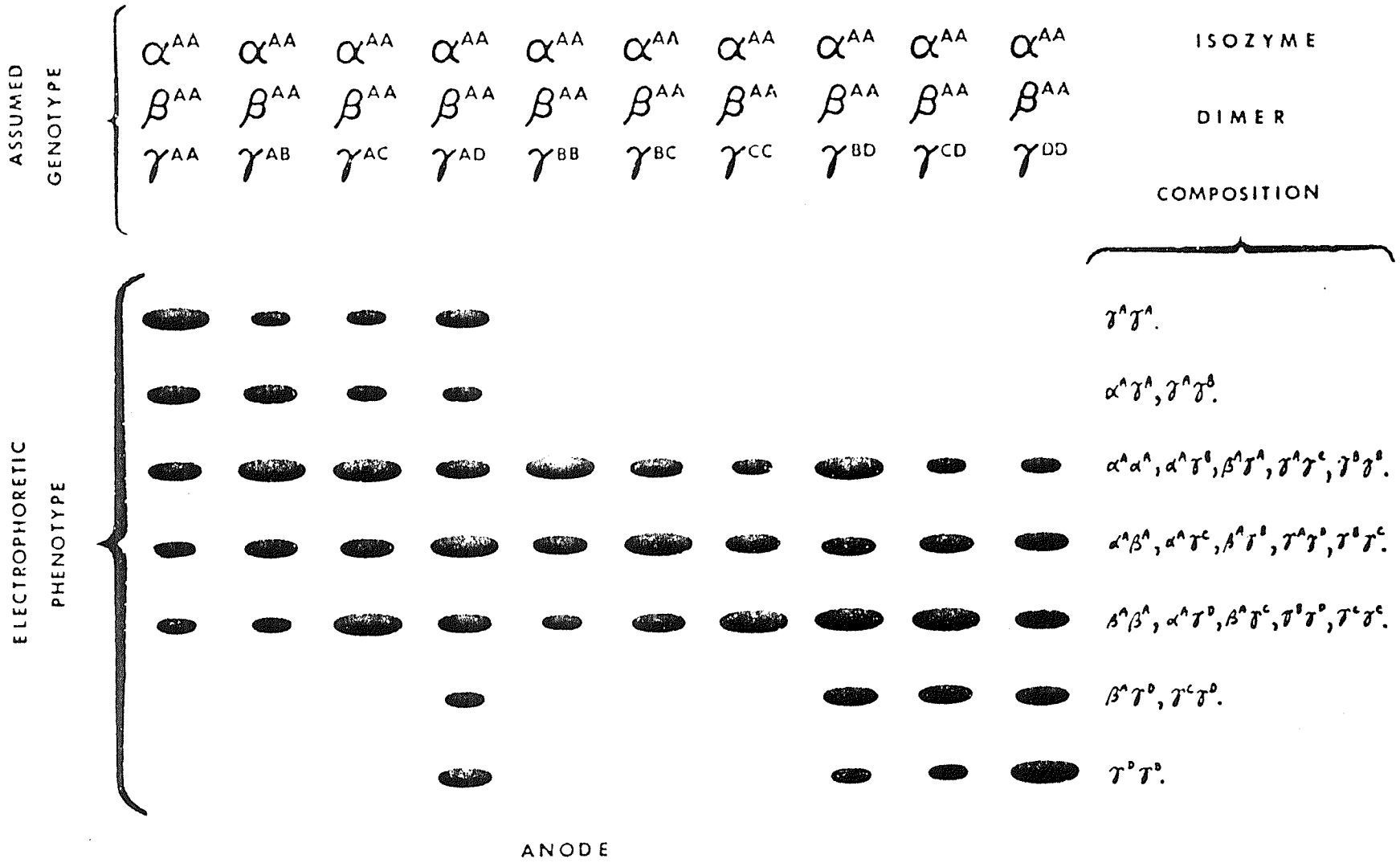
The genotype corresponding to the electrophoretic phenotype of each fish was derived from the established genetic models for LDH (Clayton and Franzin 1970) and G-3-PDH Clayton et al. (1973). The IDH genotypes were inferred from a genetic model presented in Bodaly (1977) (Fig. 3). This involves three loci, two monomorphic and one polymorphic with four alleles. Bodaly (1977) considered IDH (supernatant NADP form) in lake whitefish to function as a dimer. There is some doubt as to whether the IDH isozymes in lake whitefish are the products of two or three loci. Allendorf and Utter (1973) concluded the IDH (supernatant NADP form) isozymes in

Table 1. The isozyme systems analysed, tissues sampled and exact electrophoretic methods used in the study of lake whitefish populations from across western Canada.

Enzyme or Protein	EC No.	Locus Abbreviation	Tissues	Electrophoretic Methods
Glycerol-3-phosphate dehydrogenase (G-3-PDH)	1.1.1.8	g-3-pdh	white muscle	Clayton et al. (1973)
Lactate dehydrogenase Heart-type (LDH)	1.1.1.27	ldhH	red muscle	Clayton and Franzin (1970)
Isocitrate dehydrogenase (IDH) (NADP supernatant form)	1.1.1.42	idh	liver	Bodaly (1977)[1]
Hemoglobin			red blood cells	Lindsey et al. (1970)

[1] Liver tissue was ground with a teflon grinder with 2 volumes of 300 mg/L NADP in 0.25 M sucrose. Samples were centrifuged for 20 minutes at 25,000 g and 1°C. Buffer solutions and concentration of starch were the same as those noted in Clayton and Franzin (1970) except that NADP at 100 mg/l was added to the starch buffer, as was 3-mercapto, 1,2-propanediol (at a level of 5×10^{-3}M). The following stain solution was used: 0.15 m Bicine pH 8.5 (Na OH): 80.0 ml, sodium isocitrate monohydrate: 120 mg, $MgCl_2$ 6 H_2O: 40 mg, NADP (30 mg/ml): 0.8 ml, NBT (10 mg/ml): 1.6 ml, PMS (5 mg/ml): 0.48 ml.

Figure 3. Assumed genotype and subunit composition for isocitrate dehydrogenase (supernatant NADP form) electrophoretic phenotypes (from Bodaly 1977).


ASSUMED GENOTYPE
ELECTROPHORETIC PHENOTYPE
ISOZYME DIMER COMPOSITION
ANODE
α^{AA} β^{AA} γ^{AA}
α^{AA} β^{AA} γ^{AB}
α^{AA} β^{AA} γ^{AC}
α^{AA} β^{AA} γ^{AD}
α^{AA} β^{AA} γ^{BB}
α^{AA} β^{AA} γ^{BC}
α^{AA} β^{AA} γ^{CC}
α^{AA} β^{AA} γ^{BD}
α^{AA} β^{AA} γ^{CD}
α^{AA} β^{AA} γ^{DD}
$\gamma^{A}\gamma^{A}$.
$\alpha^{A}\gamma^{A}$, $\gamma^{A}\gamma^{B}$.
$\alpha^{A}\alpha^{A}$, $\alpha^{A}\gamma^{B}$, $\beta^{A}\gamma^{A}$, $\gamma^{A}\gamma^{C}$, $\gamma^{B}\gamma^{B}$.
$\alpha^{A}\beta^{A}$, $\alpha^{A}\gamma^{C}$, $\beta^{A}\gamma^{B}$, $\gamma^{A}\gamma^{D}$, $\gamma^{B}\gamma^{C}$.
$\beta^{A}\beta^{A}$, $\alpha^{A}\gamma^{D}$, $\beta^{A}\gamma^{C}$, $\gamma^{B}\gamma^{D}$, $\gamma^{C}\gamma^{C}$.
$\beta^{A}\gamma^{D}$, $\gamma^{C}\gamma^{D}$.
$\gamma^{D}\gamma^{D}$.

rainbow trout, Salmo gairdneri, a species in the same family as lake whitefish, were the products of two loci, one monomorphic and the other with four alleles.

Hemoglobin electrophoretic phenotypes were scored as either 'fast' or 'slow' depending on their mobility in the anodal direction on the starch gel. Lindsey et al. (1970) previously used this obvious distinction to characterize lake whitefish populations. The 'slow' class corresponds to the 'type B' figured by Tsuyuki et al. (1966b).

Previously unknown isozyme patterns of G-3-PDH were discovered in the present study. Clayton et al. (1973) demonstrated that the isozyme products of different G-3-PDH loci could be differentiated on the basis of their resistance to thermal denaturation. Samples containing the new isozyme patterns were electrophoresed concurrently with control samples, whose probable genetic base had been determined by breeding experiments. Before staining, the gel containing both the new isozyme patterns and the control was placed in a 50^{o}C hot water bath for 10 minutes. The isozymes were then stained and the relative amount denaturation noted between the new isozymes and those of known genetic base.

A large proportion of the electrophoretic data used in this study have been presented in the following publications: Franzin and Clayton (1977), Bodaly and Lindsey (1977), Bodaly (1977) and Franzin (1974). In addition, all of these authors have kindly donated additional unpublished data for use

in this thesis. The source(s) of the electrophoretic information for each population examined is given in Table 2.

Division of the Liard and Peace River Systems Into Upper and Lower Portions

For the purposes of present study the Liard River watershed is divided into upper and lower portions in the region of the Grand Canyon of the Liard (see Fig. 14). Rapids in this region were considered by McPhail and Lindsey (1970) to prevent upstream dispersal of fish species. The exact line for the division of the Liard River system is taken here to be where the Deer River (see Fig. 14) joins the Liard River. Rapids in this area (Devil's Portage) have been identified as the most impenetrable of those of the Grand Canyon of the Liard (J. Irvine, pers. comm.). The Peace River Canyon, near Hudson Hope, B.C., is used to divide the Peace River system into upper and lower portions for similar reasons.