GENETIC RELATIONSHIPS AMONG THREESPINE STICKLEBACKS *GASTEROSTEUS ACULEATUS*

by

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Abstract

Threespine sticklebacks (*Gasterosteus aculeatus*) inhabit both marine and freshwater environments along the Pacific coast of North America. In this study, sticklebacks collected from 73 locations om Vancouver Island, the Sechelt Peninsula and the lower Fraser River Valley of British Columbia, and six sites in northwestern Washington State, were assayed by starch gel electrophoresis in order to examine relationships among and between marine (trachurus) and freshwater (leiurus) populations. Six enzymes, coded for by eight genetic loci, were examined. Of these, two were monomorphic for the same allele in all populations, the remainder were polymorphic to varying degrees. Laboratory breeding studies employing both marine and freshwater fish as parents confirmed the genetic interpretation of observed variability in isozyme banding patterns. One of the monomorphic enzymes, isocitrate dehydrogenase, exhibited a sexually dimorphic isozyme pattern.

Levels of polymorphism and heterozygosity were slightly higher than average, but within the range of those characterizing other vertebrate species. In general, genotypic ratios conformed to Hardy-Weinberg expectations, and allele frequencies within populations did not shift over short time periods. Gene frequencies did not vary among sticklebacks of different sizes nor among those caught by different methods from the same population.

Gene frequencies at all polymorphic loci were significantly heterogeneous among stickleback populations. Average frequencies

at two loci (Pgm and Mdh-3) differed significantly between marine and freshwater fish. In addition, average frequencies at two other loci (Mdh-1 and Ck) were different among sticklebacks inhabiting different types of freshwater environments. Among freshwater populations the Ck^{85} and Pgm^{90} alleles displayed clinal geographic variability in frequency, possibly as a result of the differential sampling of various freshwater habitat types in different regions. Allele frequencies at three loci (Pgm, Ck and Pgi-2) differed between marine sticklebacks collected from the Strait of Georgia and those from waters off the west coast of Vancouver Island.

Calculation of Nei's genetic distance indicated that while marine populations are relatively homogeneous at electrophoretic loci, freshwater populations are highly heterogeneous. The average genetic distance between marine and freshwater populations was similar to that separating pairs of freshwater populations. The genetic distance between freshwater populations was greater between than within watersheds, but allele distributions at individual loci did not differ significantly between two watersheds.

Levels of polymorphism and heterozygosity were relatively high in populations from the ocean, large lakes and low-lying streams, and low in those from small lakes and isolated streams. Both deterministic (natural selection) and stochastic (founder effects and genetic drift) mechanisms can be invoked to explain these patterns.

Morphologically and ecologically distinct benthic and limnetic sticklebacks within single lakes, and freshwater and

marine sticklebacks within a stream, constituted genetically discrete populations. There was a striking congruence in the patterns of morphological and electrophoretic variability among the populations comprising such 'species pairs'.

The results of this study are compatible with the suggestion that freshwater populations of the study area are polyphyletic, and have arisen independently from marine sticklebacks which invaded the region since the last ice age, about 10,000 years ago.

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INTRODUCTION

Taxonomic relationships among populations of the threespine stickleback (*Gasterosteus aculeatus*) on the Pacific coast of North America have been the subject of lively debate, and even dispute (Heuts 1947, Hagen 1967, Miller and Hubbs 1969, Hagen and McPhail 1970, Bell 1976). The species exhibits two basic life history patterns. One form of *Gasterosteus*, commonly called trachurus, is an anadromous marine fish that breeds in freshwater during the spring, and the other, known as leiurus, is a permanent freshwater resident of coastal lakes and rivers.

Morphological variability within the species or "species-complex" (McPhail and Lindsey 1970), especially among freshwater populations, is extreme. Characters such as lateral plate development and number, dorsal spine number and length, gill raker number and length, ventral spine and pelvic girdle development, vertebrae number, etc. vary greatly, even over distances as short as several hundred yards (Hagen 1967, Narver 1969, Miller and Hubbs 1969, Hagen and Gilbertson 1972, Moodie 1972a, Larsen 1976). Since many of these characters have a genetic basis (Hagen 1973, Ross 1973, Hagen and Gilbertson 1973a, Avise 1976) and respond rapidly to local agents of natural selection (McPhail 1969, Hagen and Gilbertson 1973b, Kynard 1972, Moodie 1972b, Moodie *et al.* 1973, Moodie and Reimchen 1976, Bell and Haglund 1978, Gross 1978), they are of little use as taxonomic criteria. The purpose of the present study was to examine electrophoretically-detectable genetic variability in enzymatic gene frequencies, and intrepret the

findings in view of present concepts of stickleback evolution along the Pacific coast.

Fossil records for Gasterosteus on the Pacific coast of North America date back ten million years and reveal that distinctly different freshwater and marine forms existed at least as early as the Late Miocene (Bell 1976). The present range of the threespine stickleback extends from Alaska to Baja California (McPhail and Lindsey 1970). Over this range the trachurus form appears to be absent from the extreme southern areas and the leiurus form from the extreme northern areas. Presumably, populations along the British Columbia coast were established about 10,000 years ago, after the retreat of the last (Wisconsin) ice sheet. McPhail and Lindsey (1970) suggest that freshwater and marine refugia for G. aculeatus during the ice age existed to the the south (the Pacific refuge) and possibly to the north (the Bering refuge). Stickleback populations inhabiting the area encompassed by the present study, the lower Fraser River Valley, Vancouver Island and the Sechelt Peninsula, are the result of post-glacial dispersal from the southern, and possibly the northern, refugia.

The wide coastal distribution of Gasterosteus and its absence from interior areas indicate that post-glacial invasion of the North Pacific region took place through the ocean. Dispersal along the British Columbia coast was probably possible about 9000 years ago (McPhail and Lindsey 1970). Since the leiurus form has a low saltwater tolerance (Heuts 1947, Wootton 1976) it is unlikely to disperse through the sea. Therefore, the dispersing and colonizing sticklebacks must have been trachurus.

Bell (1976) states that temperature fluctuations at this time probably caused a number of north-south shifts in the range of trachurus along the coast. He suggests that freshwater leiurus populations evolved from small numbers of trachurus which invaded freshwater and then became isolated when marine conditions became unfavourable. Subsequent divergence, even in the face of probable secondary contact with marine populations, took place in these freshwater systems. This sequence gave rise to the present highly variable leiurus phenotypes. This model entails the polyphyletic evolution of freshwater sticklebacks from the marine form. Because this evolution occurred independently in different freshwater systems, morphological similarities among freshwater populations in different drainages are as likely to indicate similar selective regimes as common ancestry.

Based on this evolutionary scheme, a number of hypotheses can be generated about genetic relationships within and among stickleback populations. Making the assumption that genetic variability at the enzyme level is less subject to natural selection, and therefore more time-dependent, than are morphological traits, Bell (1976) made a series of predictions concerning the patterns of electrophoretic variability that should be revealed. He suggested that marine populations should display greater <u>intrapopulation</u> genetic variability than freshwater ones because, according to the model, they are the oldest in the region, and have had the longest time to acquire variability through mutation. In addition, the continuous marine environment facilitates gene flow, and therefore the spread of

newly generated genetic variation. Freshwater populations, on the other hand, should exhibit decreased variability owing to founder effects (the result of originating from small numbers of trachurus that perhaps possessed only some fraction of the total species variability), and subsequent stochastic events that occurred when population sizes were small (genetic drift, especially during population bottlenecks). At the same time, the greater opportunity for gene flow among marine populations should result in lower interpopulation genetic heterogeneity among trachurus than leiurus populations. This is because the restriction of gene flow between leiurus populations prevents the exchange of genetic information.

If freshwater sticklebacks all descended from marine forms, and selection has not changed allele frequencies or established new alleles in leiurus populations, then genetic loci that are monomorphic in trachurus populations should also be monomorphic in leiurus populations (Bell 1976). In contrast, loci that are polymorphic in trachurus populations may or may not be polymorphic in leiurus populations, but average frequencies should not differ between the two forms. If fixation in freshwater populations occurs at loci that are polymorphic in marine populations, then the number of freshwater populations fixed for each allele of a locus should be proportional to the frequency of that allele in marine populations.

Finally, ecologically and morphologically dissimilar populations within a freshwater drainage system should display greater electrophoretic similarity (due to their common origin) than morphologically similar populations from different drainage

systems that inhabit similar types of habitats, but presumably evolved independently from the marine form.

The purpose of the present study was to test the above predictions by measuring the molecular genetic variability in a number of marine and freshwater populations of G. aculeatus. In addition, an attempt was made to test the assumption of selective neutrality, or near-neutrality, of the observed electrophoretic heterogeneity by seeking gene-environment relationships among populations and gene-morphology relationships among certain groups of sticklebacks, and by comparing rates of morphological and electrophoretic change in an introduced stickleback population. Section I of the report contains the combined results and discussion of two sets of breeding experiments conducted to establish the genetic basis for, and inheritance patterns of, the observed electrophoretic variability. Section II consists of the results and discussion, presented separately, of measurements of genetic heterogeneity within and among the G. aculeatus populations examined.

MATERIALS AND METHODS

Nomenclature

The enzymes examined in this study, their International Union of Biochemistry (1965) numbers, and their abbreviations are listed in Table 1. Throughout the text the capitalized enzyme abbreviation (e.g. PGM) designates the enzyme itself, and the same abbreviation with only the first letter capitalized indicates the gene, or locus, coding for the enzyme. In a case of multiple loci coding for different forms of a single enzyme, the different enzymatic forms and the genes coding for them are distinguished by appending a different numeral to each (e.g. MDH-1, Mdh-1). The numeric designation of multiple enzymatic forms increases with decreasing anodal mobility. The different alleles of a single locus are distinguished by superscript numbers. A common allele of each gene is designated by a superscript 100 (e.g. Ck^{100}), other alleles by superscript numbers indicative of the anodal mobility of their enzyme products relative to that of allele 100.

Field Collections

The sticklebacks used for electrophoresis in this study were collected from sites on Vancouver Island, the Sechelt Peninsula and the lower Fraser River Valley in British Columbia. In addition, several sites in northwestern Washington were

Table 1. The enzymes, buffer systems and staining solutions used in this study. Enzymes are numbered in accordance with the International Union of Biochemistry (1965). Stains for PGI, PGM, LDH and IDH are mixed in 100 ml of 50 mM Tris adjusted to pH 7.1 with HCl. The stain for MDH is mixed in 100 ml of gel Buffer I.

Enzyme	Abbreviation	Buffer	Stain Components
Creatine Kinase (2.7.3.2)	CK	I	General protein stain: 0.1% Amido Black 10B and 0.1% Napthol Blue Black in a 1:4:5 mixture of acetic acid: methanol: water. Destain: 1:4:5 acetic acid: methanol: water.
Isocitrate Dehydrogenase (NADP) (1.1.1.42)	IDH	II or III	50 mg DL-Na-isocitrate 10 mg NADP 15 mg NBT (or MTT) 5 mg PMS 50 mg $MgCl_2$
Lactate Dehydrogenase (1.1.1.27)	LDH	I	20 ml 0.5 M DL-Na-lactate 10 mg NAD 15 mg NBT (or MTT) 5 mg PMS
Malate Dehydrogenase (1.1.1.37)	MDH	II or III	20 ml 0.5 M DL-Na-malate 10 mg NAD 15 mg NBT (or MTT) 5 mg PMS
Phosphoglucoisomerase (5.3.1.9)	PGI	I	50 mg Na-fructose 6-phosphate 100 units G6PDH 10 mg NADP 50 mg $MgCl_2$ 15 mg NBT (or MTT) 5 mg PMS

Phosphogluco-mutase (2.7.5.1)	PGM	I	200 mg K-glucose-1-phosphate 100 units G6PDH 10 mg NADP 50 mg $MgCl_2$ 15 mg NBT (or MTT) 5 mg PMS

G6PDH	Glucose-6-phosphate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	p-Nitro tetrazolium blue
MTT	3-(4,5-dimethyl Thiazolyl-2)-2,5-diphenyl tetrazolium bromide
PMS	Phenazine methosulfate

sampled. A complete list of the collection sites and their assigned abbreviations, date(s) sampled and longitudinal and latitudinal co-ordinates are given in Appendix I. The 79 sites sampled include lakes, streams, swamps and marine habitats. An attempt was made to collect at least 40 fish from each site, but this was not always possible (Table 8).

Usually, minnow traps, a long-handled dip-net or a pole seine (a 1.5 by 1 m net, with 0.6 cm mesh, supported and drawn through the water by two 2 m poles) were used for capture; however, sticklebacks were also collected in 15.5 or 31 m beach seines and, from the ocean, in an otter trawl. Captured fish were bagged in water and immediately frozen on dry ice. In the laboratory, samples were stored at -20C until used. The enzymes examined retained activity for time periods up to and exceeding one year. However, in most cases, samples were electrophoresed within four months of collection.

Crossing Techniques

In June 1977 male and female trachurus in breeding condition were seined from regions 2 km upstream from the mouth of the Little Campbell River and transported to the laboratory. Crosses were made by using testes excised from males and minced in water to fertilize eggs that had been gently extruded from gravid females. To maximize the number of parental genetic combinations, milt from a single male was sometimes used to fertilize egg clutches from two different females and, similarly, an egg clutch from one female was sometimes divided

in half and fertilized with milt from different males. All fish employed as parents were frozen for subsequent electrophoresis.

Eggs and newly-hatched young were incubated initially in 682 ml (24 oz) glass jars of oxygenated fresh water held at room temperature (18C-23C). Two weeks after hatching, broods were transferred to 22.7 l (five-gallon) aquaria at 23C. As the fish grew, broods were subdivided among more tanks to prevent crowding. The young were fed newly-hatched brine shrimp nauplii (Artemius) twice daily for the first two months and frozen brine shrimp thereafter. Offspring of these crosses suffered high mortality throughout their existence; as eggs (fungal infection), at hatching, and upon transfer to aquaria. Survivors (representing 16 of 22 original crosses) were frozen for electrophoresis at intervals from 6 weeks to 6 months after hatching.

Better sample sizes were obtained from a second set of crosses carried out in April 1979. Leiurus sticklebacks collected 15 km upstream from the mouth of the Little Campbell River were employed as parents. Four males placed in separate 22.7 l aquaria built nests and courted gravid females that were introduced into the tanks. Spawning occurred naturally; one male fertilized eggs from two females and the other three males each fertilized eggs from one female. Female parents were frozen immediately after egg deposition. Males were left to care for the eggs and young until one week after hatching, and then removed and frozen.

The young were maintained at room temperature (19C-23C) on a diet of brine shrimp nauplii and, after several weeks, chopped

Tubifex worms. One week after emergence from the nest each brood was subdivided among several aquaria. Samples of young were removed and frozen for electrophoresis at four, eight and sixteen weeks after hatching. Mortality in these crosses was low, and it is likely that the fish so obtained represented random samples of entire broods.

Sample Preparation

The enzymes examined in this study are all present in *Gasterosteus* muscle tissue. To aid tissue removal, sticklebacks frozen in water were partially thawed, separated, laid out on a tray and refrozen. For sticklebacks over 2 cm in length, samples were sliced from either the left or right side of the caudal region. The entire caudal region (both sides) was used in fish between 1 and 2 cm in length, and the whole fish was used when its length was less than 1 cm. When entire fish were used, enzymes from other tissues stained, but these were easily distinguished from muscle enzymatic forms.

The frozen tissue samples were placed in 13 x 75 mm plastic test tubes with an equal volume of distilled water, and the test tube rack was kept on ice. Samples were then ground thoroughly with a glass rod and refrozen. These tubes were stored for up to a week prior to electrophoresis. For use, they were centrifuged for 6 min. at 3000 rpm, and kept on ice while the gels were loaded.

Gel Preparation

The electrophoretic equipment and techniques employed were adopted, with slight modifications, from those described in detail by May (1975). The gel mold consisted of four plexiglass strips clamped to a flat plexiglass sheet, 26.7 cm (10.5 in) x 17.8 cm (7 in) x 0.64 cm (0.25 in). The strips forming the length of the mold were 21.6 cm (8.5 in) x 1.9 cm (0.75 in) x 1.3 cm (0.5 in) and edging the width were 17.8 cm (7 in) x 1.9 cm (0.75 in) x 1.3 cm (0.5 in).

Gels consisted of 45.5 g of Electrostarch (Lot 307, Otto Hiller Electrostarch Co., Madison, Wis.) in 350 ml of buffer. Approximately one-quarter of the buffer was added to the starch in a 500 ml flask and swirled to dissolve the starch. The remaining buffer was heated to boiling and, with constant swirling, added to the flask containing the starch mixture. The flask contents were heated to vigorous boiling, degassed with an aspirator for one minute and finally poured into a gel mold. The gel was ready for use after cooling at room temperature for one-half hour.

Buffer Systems

Three buffer systems were used:

(I) Electrode buffer: 60 mM lithium hydroxide, 300 mM boric acid, pH 8.1.

Gel buffer: 30 mM Tris, 5mM monohydrate citric acid, pH 8.5.

Gels consisted of 99% gel buffer and 1% electrode buffer. (Ridgway et al. 1970).

(II) Electrode buffer: 40 mM monohydrate citric acid adjusted to

pH 6.1 with N-(3-Aminopropyl)-morpholine.
Gel buffer: a 1 in 20 dilution (in distilled water) of electrode buffer.
(Clayton and Tretiak 1972).

(III) Electrode buffer: 135 mM Tris, 45 mM monohydrate citric acid, pH 7.0.
Gel buffer: a 1 in 15 dilution (in distilled water) of the electrode buffer.
(Ayala et al. 1972).

Electrophoresis

For electrophoresis, the two long plexiglass strips were removed from the gel form and a cut made along the length of the gel approximately 3 cm from one exposed edge. This cut separated the gel into two pieces of unequal size. Filter paper (Schleicher and Schuell, grade 470) wicks, 0.2 x 1 cm in size, were dipped in the muscle tissue samples and stationed vertically along the cut gel surface. At regular intervals wicks dipped in dilute red food colouring were inserted as markers. A total of 40 wicks, representing muscle samples from 40 different fish, were loaded on one gel. Then the two gel pieces, with wicks sandwiched between, were pressed firmly back together and the gel covered with plastic wrap. This wrap was folded back to expose approximately 2 cm of gel along each long edge. The plexiglass plate supporting the gel was placed between two buffer trays containing platinum wire electrodes. Absorbent

cloths were used to establish contact between the exposed gel edges and the electrode buffer in the trays. The electrodes then were connected to a power source in such a way that the gel edge nearest the wicks formed the cathodal (negative) end.

After ten minutes of electrophoresis the wicks were removed and the two gel pieces pressed firmly together. The entire gel and buffer trays then were covered with another sheet of plastic wrap and an ice pack was placed on the gel. All of the enzymes studied, and the food colouring marker used, migrated anodally. For buffer systems I and III, 200-250 volts (40-60 milliamps) were applied until the red dye had migrated to within 1.5 cm of the anodal gel edge (4-5 hours). For buffer system II, 60 milliamps (200-300 volts) were applied until the red dye marker reached the anodal gel edge (about 4 hours).

Enzyme Assay

After electrophoresis the remaining two plexiglass strips were removed from the gel form. The cathodal gel strip was discarded. Thin plexiglass strips, 2 mm thick, were stacked along the two long edges of the gel, and used as guides along which nylon thread was drawn to slice the gel into layers. The top and bottom layers were discarded, and the remaining slices placed in separate staining trays. These slices were incubated at 37C in the specific and general (for CK) enzyme stains listed in Table 1. Most of the stains are from, or adapted from, recipes of Shaw and Prasad (1970). Gels in specific stains were incubated until bands were clear (up to one hour) and then

scored. The gel in the general protein stain was incubated for 20 minutes, then rinsed several times with water and left in the destaining solution (Table 1) overnight. It was scored the following day.

SECTION I. DESCRIPTION AND INHERITANCE OF *GASTEROSTEUS* ISOZYME PATTERNS

Electrophoretic examination of variability at single loci coding for enzymatic proteins provides a simple and accurate method of determining genetic differentiation both within and between species. Electrophoresis provides minimal estimates of the genetic distance separating populations because only about one-third of all possible point mutations in DNA nucleotide sequences will produce electrophoretically-detectable changes in the protein products (Shaw 1965). However, the codominant expression (i.e. lack of dominance) of alleles that can be detected enables the precise assessment of their gene and genotype frequencies.

Care must be taken in the interpretation of heterogeneity in isozyme patterns. Sources of variation other than simple allelic differences among individuals include changes in gene expression during development, variability due to environmental factors such as temperature and salinity, and *in vitro* changes brought about by the conditions of sample storage and extraction procedures (Allendorf and Utter 1979). Usually banding patterns show typical Hardy-Weinberg proportions and this is often considered sufficient evidence for the underlying genetic basis of the variability. However, breeding studies are required for absolute confirmation.

This section consists of description of the observed *Gasterosteus* isozyme patterns and their comparison with those of other species. Isozyme patterns of progeny of the Little

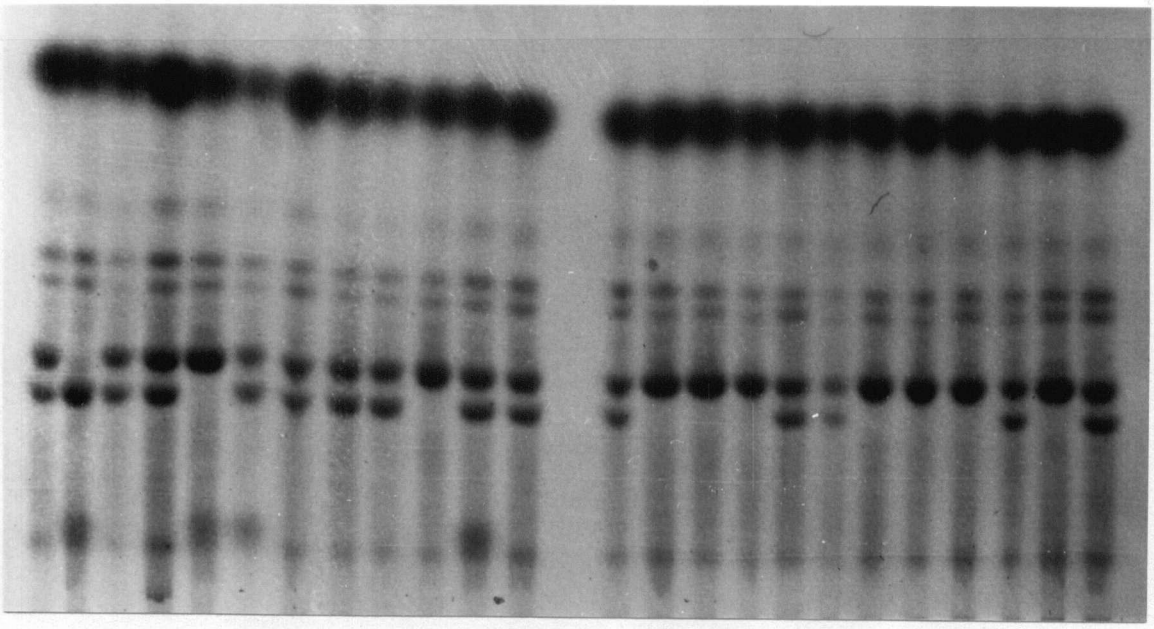
Campbell crosses are tested for conformation to predicted genotypic proportions based on a Mendelian interpretation of variability in their parents. In all cases, isozyme patterns of the young resembled those of the adults. This indicates an absence of either ontogenetic or environmental influences on the patterns. The allelic interpretation of variability was confirmed for all enzymes but one (IDH).

Creatine Kinase

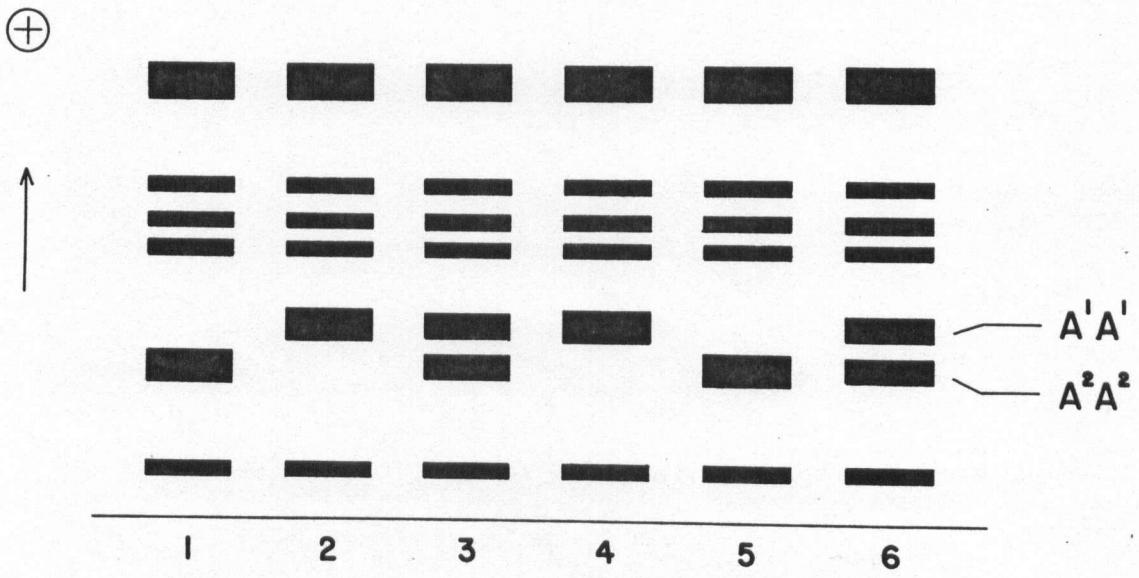
Phenotypic variability in Gasterosteus CK patterns was scored from gels stained with the general protein stain Amido Black 10B (Michiel 1977). The isozymes monitored were by far the darkest staining bands on the gel. This is consistent with the demonstrations by Gosselin-Rey et al. (1968) and Gosselin-Rey and Gerday (1970) that creatine kinase constitutes a large proportion (16% in carp) of the white muscle cell proteins in fish. In addition, the CK banding pattern of sticklebacks (Fig. 1) conforms with that found in other teleosts by specific staining methods (Ferris and Whitt 1978).

Sticklebacks possess either one or both of two CK isozyme bands (A^1A^1 and A^2A^2 in Fig. 1). Results of the Little Campbell leiurus crosses (Table 2) indicate that this variability is under the control of a single autosomal locus with two alleles. Individuals displaying the A^1A^1 band are homozygous for an allele termed Ck^{100} (FF in Table 2), those displaying the A^2A^2 band are homozygous for an allele termed Ck^{85} (SS) and those displaying both bands are Ck^{100}/Ck^{85} (FS) heterozygotes. The

Figure 1. Threespine stickleback CK isozymes on a gel stained with a general protein dye. Individuals homozygous for Ck^{100} (nos. 1 and 3) display the A^1A^1 band, those homozygous for Ck^{85} (no. 4) display the A^2A^2 band, and Ck^{100}/Ck^{85} heterozygotes (nos. 2 and 5) display both bands.



CK



homozygous and heterozygous leiurus parents of every cross produced progeny displaying Mendelian proportions of genotypes consistent with control by a single autosomal gene with two alleles. The Little Campbell trachurus population is monomorphic for the Ck^{ss} allele, and all parents and progeny of crosses involving these fish displayed only the A^2A^2 band (Table 2).

Creatine kinase is a dimeric enzyme (Dawson et al. 1967, Gosselin-Rey and Gerday 1970) and, as such, should exist in three isozyme forms in individuals heterozygous at a Ck locus. The expected banding pattern based on a binomial association of the two peptides produced consists of a 1:2:1 ratio of homodimer:heterodimer:homodimer. In vertebrates other than fish, heterozygotes for muscle creatine kinase (commonly referred to as CK-A) exhibit just such a 3-banded pattern (Ferris and Whitt 1978). However, as first noted by Scopes and Gosselin-Rey (1968) for carp (Cyprinus carpio) and Tilapia spp., teleosts heterozygous for muscle creatine kinase typically possess only two electrophoretically distinct isozyme forms, and consequently display a 2-banded pattern similar to that of Gasterosteus. Ferris and Whitt (1978) extended the examination of CK-A phenotypes to species belonging to seven orders of bony fishes and found the 2-banded pattern to be characteristic of individuals heterozygous at any one Ck-A locus in all species that showed genetic variability. They demonstrated that muscle CK of fishes exists only in homodimeric forms; in heterozygotes the expected heterodimer of intermediate mobility is not formed. Their findings indicated that there exists a "spatial and/or temporal isolation of the synthesis and/or assembly" of creatine

Table 2. Inheritance of Ck alleles in Little Campbell River trachurus and leiurus sticklebacks. In both sets of crosses the faster allele (F) is Ck^{100} and the slower allele (S) is Ck^{85}. Parental genotypes in brackets were inferred from progeny genotypic ratios.

Parental Genotypes	No. of Crosses	Progeny FF	Progeny FS	Progeny SS	Parental Genotypes	No. of Crosses	Progeny FF	Progeny FS	Progeny SS
Trachurus									
♂ SS ♀ SS	13	0	0	198	♂ (SS) ♀ (SS)	3	0	0	49
Leiurus									
♂ FF ♀ FS	1	30	28	0	♂ FS ♀ FS	1	7	21	13
♂ FF ♀ FS	1	19	27	0	♂ SS ♀ FS	1	0	26	32
♂ FS ♀ FS	1	20	36	19					

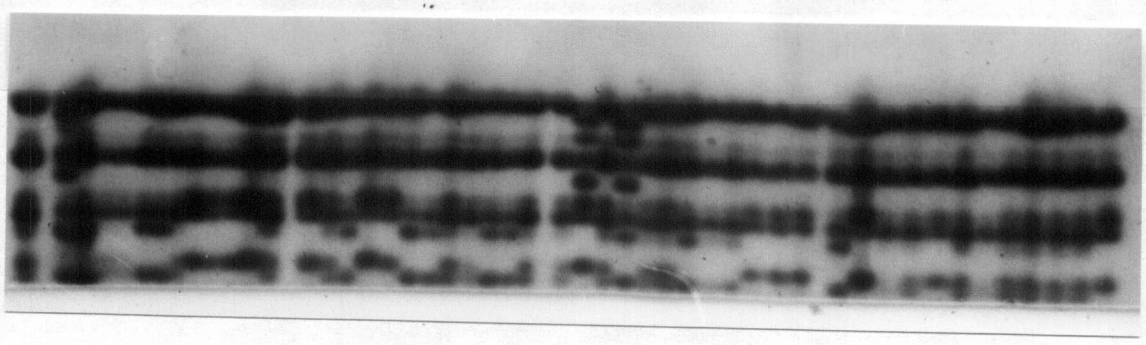
kinase peptides coded for by the two Ck alleles located on homologous chromosomes in muscle cells. The evolutionary significance of this widespread restriction of subunit interaction in the muscle CK of bony fishes is not known (Ferris and Whitt 1978).

Malate Dehydrogenase

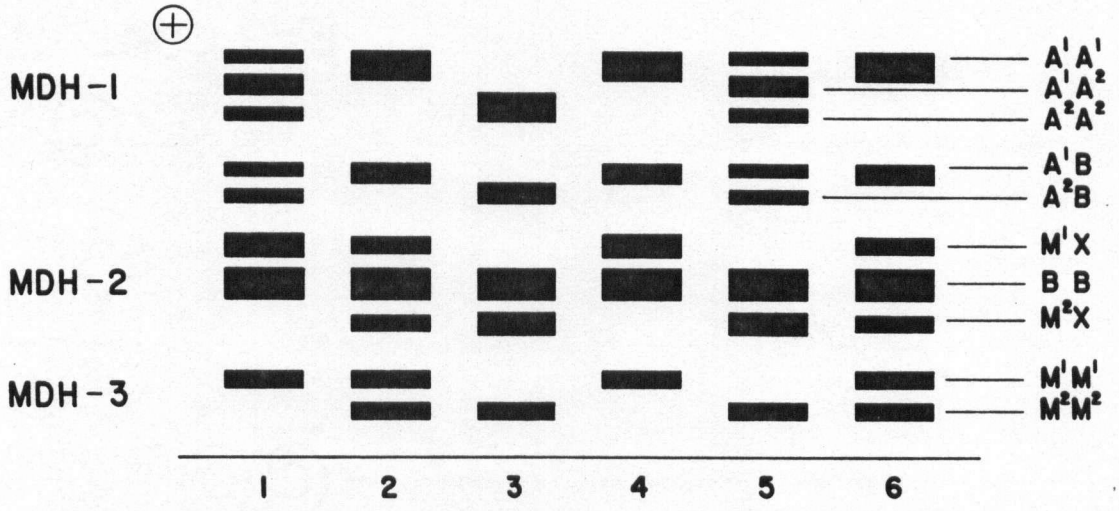
The relatively complex pattern of Gasterosteus MDH (Fig. 2) is consistent with the presence, in vertebrates, of genetically distinct cytoplasmic (supernatant) and mitochondrial malate dehydrogenases (Thorne et al. 1963, Davidson and Cortner 1967, Wheat and Whitt 1971). Moreover, many organisms, including numerous teleost species, possess two or more genes coding for supernatant malate dehydrogenases of differing electrophoretic mobilities (Bailey et al. 1970, Whitt 1970, Wheat and Whitt 1971, Clayton et al. 1973). Genetically distinct multiple mitochondrial forms may also exist in some species (Kitto et al. 1966).

MDH is a dimer, and the association of polypeptide products of different alleles of a single genetic locus, or two different loci, to form functional heterodimeric enzymes results in the production of numerous isozymes. The dimerization of peptides coded for by separate supernatant loci is common (Bailey et al. 1970, Clayton et al. 1971), and in vitro techniques such as freezing and thawing tissue enhance the association of peptides coded for by supernatant and mitochondrial loci within a species (Chilson et al. 1966), or two supernatant or mitochondrial

Figure 2. The MDH isozyme banding patterns of threespine sticklebacks. Individuals homozygous at the Mdh-1 locus for the $Mdh\text{-}1^{100}$ allele (nos. 2, 4 and 6) possess the A^1A^1 band, those homozygous for $Mdh\text{-}1^{82}$ (no. 3) possess the A^2A^2 band, and $Mdh\text{-}1^{100}/Mdh\text{-}1^{82}$ heterozygotes (nos. 1 and 5) possess all three A^1A^1, A^1A^2 and A^2A^2 bands.
Individuals homozygous at the Mdh-3 locus for $Mdh\text{-}3^{100}$ (nos. 1 and 4) possess the M^1M^1 and M^1X bands, $Mdh\text{-}3^{55}$ homozygotes (nos. 3 and 5) possess the M^2M^2 and M^2X bands, and $Mdh\text{-}3^{100}/Mdh\text{-}3^{55}$ heterozygotes (nos. 2 and 6) possess M^1M^1, M^2M^2, M^1X and M^2X.
All sticklebacks possess the BB band, possibly the homodimeric product of a monomorphic Mdh-2 locus. The A^1B and A^2B bands represent hybrid isozymes.



MDH


⊕
MDH-1
MDH-2
MDH-3
A¹A¹
A¹A²
A²A²
A¹B
A²B
M¹X
B B
M²X
M¹M¹
M²M²
1
2
3
4
5
6

peptides from different species (Chilson et al. 1966, Wheat and Whitt 1971, Clayton et al. 1973).

The MDH pattern of Gasterosteus (Fig. 2) is best explained in terms of three structural loci, termed Mdh-1, Mdh-2 and Mdh-3 in order of decreasing mobility of enzyme products. The most anodal (Mdh-1) and most cathodal (Mdh-3) of the loci are significantly polymorphic, while Mdh-2 is apparently virtually monomorphic in all populations.

Individuals homozygous for the most common Mdh-1 allele ($Mdh\text{-}1^{100}$) possess the fast-migrating homodimeric isozyme labelled A^1A^1 in Fig. 2. Individuals heterozygous at this locus for the $Mdh\text{-}1^{100}$ allele and a variant allele $Mdh\text{-}1^{82}$ display the characteristic 3-banded phenotype produced by a heterozygous locus coding for a dimeric enzyme. The bands labelled A^2A^2 and A^1A^2 represent the variant homodimeric and the heterodimeric isozymes respectively. The relative intensity of staining of the A^1A^1, A^1A^2, A^2A^2 bands is 1:2:1 as expected.

Ratios of progeny phenotypes in the Little Campbell crosses support the diallelic, single locus interpretation of variability in this region (Table 3). Crosses in which both parents were homozygous for the $Mdh\text{-}1^{100}$ allelle (FF) produced all FF progeny. Crosses in which one parent of either sex was heterozygous for the $Mdh\text{-}1^{100}$ and $Mdh\text{-}1^{82}$ alleles (FS), and the other homozygous FF, produced one-half FF and one-half FS progeny as expected. Unfortunately, the low frequency of the $Mdh\text{-}1^{82}$ allele in both Little Campbell populations precluded the opportunity of using an $Mdh\text{-}1^{82}$ homozygote in the lab crosses, but such individuals were present in other populations. Their

electrophoretic pattern in the MDH-1 region consisted of only the A^2A^2 homodimeric isozyme (Fig. 2).

The bands labelled A^1B and A^2B in Fig. 2 represent heterodimeric hybrid isozymes formed by the association of peptide products of the Mdh-1 locus and a second, more cathodal Mdh-2 locus to be discussed below. Their hybrid nature is indicated by the complete correlation of their phenotypic variability with genotypic variation at the Mdh-1 locus. Thus an $Mdh\text{-}1^{100}$ homozygote possesses only one MDH-1 peptide (A^1) to combine with an MDH-2 peptide (B), and one hybrid isozyme (A^1B) results. An $Mdh\text{-}1^{100}/Mdh\text{-}1^{82}$ heterozygote possesses two MDH-1 peptides (A^1 and A^2) to combine with the MDH-2 peptide B, and two isozymes result (A^1B and A^2B). An $Mdh\text{-}1^{82}$ homozygote possesses only the A^2B hybrid isozyme (Fig. 2).

Next most easily accounted for in genetic and structural terms are the cathodal isozymes labelled M^1M^1 and M^2M^2. Variability in this region was found to be under the control of a single autosomal locus termed Mdh-3, individuals homozygous for the $Mdh\text{-}3^{100}$ allele possessed the M^1M^1 isozyme only, while individuals homozygous for the $Mdh\text{-}3^{55}$ allele possessed the M^2M^2 isozyme. Heterozygotes possessed both M^1M^1 and M^2M^2 isozyme bands. Such a two, rather than 3-banded pattern in the heterozygote is indicative of monomeric rather than dimeric protein structure. It is possible that bands M^1M^1 and M^2M^2 are *in vitro* dissociated monomeric peptides coded for by the two alleles, and that *in vivo* they combine to form homo- and heterodimers. Alternatively, the peptides produced by the $Mdh\text{-}3^{100}$ and $Mdh\text{-}3^{55}$ alleles may only associate to form homodimers,

and a functional heterodimeric isozyme of intermediate mobility is not produced (as with CK). A final possibility is that M^1M^1 and M^2M^2 are actually heterodimers, formed by the hybridization of peptides from a locus cathodal to the origin (and hence not observed on the gel) and a polymorphic anodal Mdh-3 locus whose products coincide with the products of the Mdh-2 locus (see below). At any rate, results of the Little Campbell trachurus and leiurus crosses confirm that the variability is due to two alleles of a single autosomal gene (Table 4). All possible combinations of homozygous Mdh-3^{100} (FF), homozygous Mdh-3^{55} (SS), and heterozygous Mdh-3^{100}/Mdh-3^{55} (FS) parental genotypes were achieved in these crosses, and in every case progeny genotypes and ratios conformed with Mendelian expectations.

Phenotypic variability in the gel region containing the M^1X, BB and M^2X bands (Fig. 2) directly reflects genotypic variability at the Mdh-3 locus. Thus, individuals homozygous for Mdh-3^{100} and possessing the M1M^1 band also display the M^1X and BB bands. Mdh-3^{55} homozygotes, with the M^2M^2 band, display the M^2X and BB bands. Mdh-3 heterozygotes, in addition to the M^1M^1 and M^2M^2 bands, possess all three M^1X, BB and M^2X bands. The relative staining intensity of the 3-banded phenotype is 1:2+:1. The relative staining intensity of the BB and whichever other band possessed by a 2-banded individual is 1+:1. The simplest explanation for the bands of this region is that the central, dark-staining BB band represents the homodimeric isozyme of a third Mdh locus (Mdh-2). Variability at this Mdh-2 locus might be difficult to score because of the proximity of the M^1X and M^2X bands on the gel. However, like the variability at the Mdh-1

Table 3. Inheritance of Mdh-1 alleles in Little Campbell River trachurus and leiurus sticklebacks. In both sets of crosses the faster allele (F) is Mdh-1^{100} and the slower allele (S) is Mdh-1^{82}. Parental genotypes in brackets were inferred from progeny genotypic ratios.

Parental Genotypes	No. of Crosses	Progeny FF	Progeny FS	Progeny SS	Parental Genotypes	No. of Crosses	Progeny FF	Progeny FS	Progeny SS
Trachurus									
♂ FF ♀ FF	11	119	0	0	♂ FS ♀ FF	1	27	36	0
♂ (FF) ♀ (FF)	3	49	0	0	♂ FF ♀ FS	1	8	8	0
Leiurus									
♂ FF ♀ FF	4	224	0	0	♂ FF ♀ FS	1	30	29	0

Table 4. Inheritance of Mdh-3 alleles in Little Campbell River trachurus and leiurus sticklebacks. In both sets of crosses the faster allele (F) is Mdh-3^{100} and the slower allele (S) is Mdh-3^{55}. Parental genotypes in brackets were inferred from progeny genotypic ratios.

Parental Genotypes		Progeny			Parental Genotypes		Progeny		
Male	Female	FF	FS	SS	Male	Female	FF	FS	SS
Trachurus									
(1) SS	(1) SS	0	0	16	(8) SS	(10) SS	0	0	10
(2) FS	(2) FF	21	22	0	(9) FF	(9) FS	3	3	0
(3) FS	(3) FS	1	5	4	(9) FF	(10) SS	0	11	0
(4) FS	(4) SS	0	7	3	(10) SS	(11) SS	0	0	10
(5) (SS)	(5) (FS)	0	11	4	(10) SS	(12) SS	0	0	7
(6) SS	(7) SS	0	0	10	(11) FS	(12) SS	0	5	5
(7) FS	(8) SS	0	9	11	(12) (SS)	(13) FS	0	7	3
(8) SS	(9) FS	0	7	4					
Leiurus									
(13) FS	(15) FF	33	26	0	(15) FF	(18) FF	42	0	0
(13) FS	(16) FF	26	21	0	(16) FF	(19) FF	58	0	0
(14) FF	(17) FF	77	0	0					

locus, variability at the Mdh-2 locus should result in additional forms of hybrid A^1B (and A^2B) isozymes. In fact, extra bands cathodal to both the BB and A^1B (or A^2B) isozymes were observed in some populations. This putative Mdh-2 variability was not monitored because it was rare and difficult to interpret.

The direct correspondence of the presence of M^1X and M^2X with the presence of M^1M^1 and M^2M^2 indicates that M^1X and M^2X are isozymes containing the peptides coded for by the Mdh-3 locus. One possible explanation is that while the M^1M^1 and M^2M^2 bands represent the monomeric peptides coded for by Mdh-3, M^1X and M^2X represent the homodimeric associations of these peptides and a heterodimer of intermediate mobility coincides with, and therefore cannot be distinguished from, the BB isozyme. The problem with this interpretation is that the sieve-like properties of starch gel should allow the smaller monomers to migrate more quickly, and therefore farther toward the anode, than the dimers. More likely, if M^1X and M^2X actually are homodimeric isozymes formed by peptides of the polymorphic Mdh-3 locus, then M^1M^1 and M^2M^2 are heterodimers composed of one peptide from the Mdh-3 locus (M^1 or M^2) and one peptide from a cathodal locus, Mdh-4, that is not visible on the gel. Thus, variability at the Mdh-3 locus can be measured either by scoring variability in M^1X and M^2X or variability in M^1M^1 and M^2M^2.

Gasterosteus muscle tissue was not subjected to subcellular fractionation to determine the origin (mitochondrial or cytoplasmic) of the various MDH isozymes. However, the close similarity of the stickleback pattern to that described for

certain other teleosts permits some tentative classification.

In general, mitochondrial MDH is more cathodal than supernatant MDH (Bailey et al. 1970, Clayton et al. 1973), although exceptions are known in the killifish, Fundulus heteroclitus (Whitt 1970) and Pacific yellow-fin tuna, Neothunnus macropterus (Kitto and Lewis 1967). Vertebrate mitochondrial MDH is characterized by its relatively high thermolability (Kitto and Lewis 1967). Observation of mitochondrial MDH activity is dependent upon mitochondrial rupture, and its activity is thus reduced or absent in tissue samples not ground or frozen prior to electrophoresis. Finally, no mitochondrial MDH can be detected by electrophoretic techniques in the livers of at least some fish species, such as the walleye Stizostedion vitreum vitreum and sauger S. canadense (Clayton et al. 1973) and the saury Cololabis saira (Numachi 1970). The slow-migrating M^1M^1 and M^2M^2 isozymes coded for by the Mdh-3 locus apparently fulfil these criteria. They tend to be cathodal and their activity was reduced in samples ground, but not frozen, before electrophoresis and in samples inadequately cooled during electrophoresis. Moreover, liver tissue samples did not display the M^1M^1 or M^2M^2 isoenzyme bands.

The anodal isoenzyme products of the Mdh-1 locus (A^1A^1 and A^2A^2), and intermediate product of the Mdh-2 locus (BB), are likely supernatant MDH. The ready association of peptides from the two loci to form hybrid dimers is indicative of the relative lack of evolutionary divergence characteristic of multiple supernatant loci (Bailey et al. 1970). The coincident electrophoretic mobilities of a supernatant MDH (such as

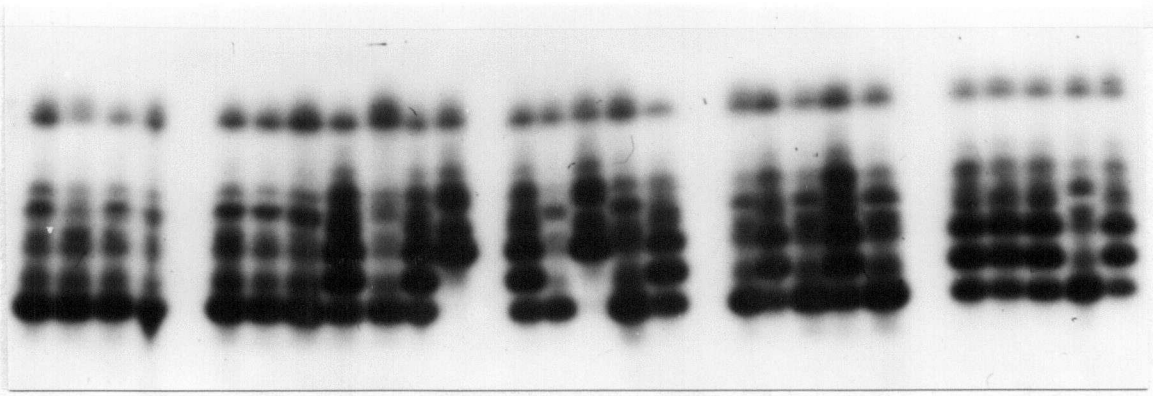
stickleback BB is hypothesized to be) and mitochondrial forms (such as stickleback M^1X and M^2X may be) has been observed before (Clayton et al. 1973). In fact, the entire Gasterosteus pattern as described and interpreted here is strikingly similar to those described by Clayton et al. (1973) for walleye and sauger. Like sticklebacks, these species possess two supernatant loci, one highly polymorphic and coding for the most anodal isozymes (like stickleback Mdh-1), and one monomorphic and coding for an isozyme of intermediate mobility (like Mdh-2). In walleye, the mitochondrial enzymes are cathodal to all supernatant forms (as are the stickleback M^1M^1 and M^2M^2 bands), while the sauger possessed a mitochondrial isozyme that coincided in electrophoretic mobility with the most cathodal isozyme of supernatant MDH (as do M^1X and M^2X).

Phosphoglucoisomerase

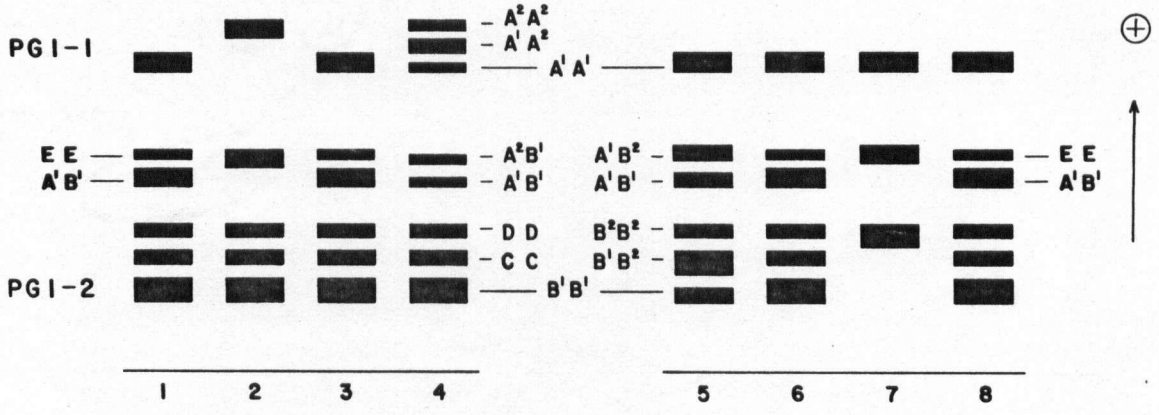
The multi-banded electrophoretic pattern of Gasterosteus PGI (Fig. 3) is similar to that exhibited by other bony fishes (Avise and Kitto 1973). Two structural genes code for PGI peptides of differing electrophoretic mobilities, Pgi-1 for the faster-migrating one and Pgi-2 for the slower. PGI is a dimer, and the bands A^1A^1 and B^1B^1 in Fig. 3 represent the two homodimeric isozymes produced by the most common alleles at each of the loci ($Pgi\text{-}1^{100}$ and $Pgi\text{-}2^{100}$ respectively). The band A^1B^1 represents the heterodimeric isozyme of intermediate mobility.

The light-staining bands CC and DD anodal to the B^1B^1 isozyme band, and EE anodal to the A^1B^1 isozyme band, are

Figure 3. The threespine stickleback PGI isozyme banding patterns. Individuals homozygous at the Pgi-1 locus for Pgi-1^{100} (nos. 1, 3 and 5 - 8) display the A^1A^1 band, those homozygous for Pgi-1^{105} (no. 2) display the A^2A^2 band, and Pgi-1^{100}/Pgi-1^{105} heterozygotes (no.4) display the A^1A^1, A^1A^2 and A^2A^2 bands.
Sticklebacks homozygous at the Pgi-2 locus for Pgi-2^{100} (nos. 1 - 4, 6 and 8) display the B^1B^1, CC and DD bands, those homozygous for Pgi-2^{147} (no. 7) display the B^2B^2 band, and Pgi-2^{100}/Pgi-2^{147} heterozygotes (no. 5) display the B^1B^1, B^1B^2 and B^2B^2 bands.
The A^1B^1, A^1B^2, A^2B^1 and A^2B^2 bands represent hybrid isozymes possessing one peptide from each of the Pgi-1 and Pgi-2 loci.



PGI


PGI-1
PGI-2
E E
A'B'
A²A²
A'A²
A'A'
A²B'
A'B'
D D
C C
B'B'
A'B²
A'B'
B²B²
B'B²
E E
A'B'
⊕
1
2
3
4
5
6
7
8

'satellite bands' (Avise and Kitto 1973). These enzymes are not genetically distinct from the B^1B^1 and A^1B^1 isozymes, but instead likely result from the oxidation of various PGI sulfhydryl groups (Noltmann 1975). They are characteristic of the electrophoretic patterns of PGI (Avise and Kitto 1973, Gracy 1975) and other enzymes (Turner et al. 1975, Dawson and Green 1975).

The most common variant allele at the Pgi-2 locus, $Pgi\text{-}2^{147}$, codes for a peptide that in homodimeric form (B^2B^2) coincides in electrophoretic mobility, and hence gel position, with the DD band. The heterodimeric isozyme of a $Pgi\text{-}2^{100}/Pgi\text{-}2^{147}$ heterozygote (B^1B^2) migrates at the same rate as the CC band (Fig. 3). However, the characteristic 1:2:1 ratio of staining intensity exhibited by the B^1B^1, B^1B^2, B^2B^2 bands in the heterozygote readily distinguish it from the $Pgi\text{-}2^{100}$ homozygote possessing the B^1B^1, CC, DD bands of identical mobilities but different staining intensity ratios (i.e. the CC and DD bands stain distinctly less darkly than the B^1B^1 band).

The presence of different alleles at the Pgi-2 locus in $Pgi\text{-}2^{100}/Pgi\text{-}2^{147}$ heterozygotes results in the production of a second hybrid isozyme A^1B^2 which is anodal to A^1B^1. The position of the A^1B^2 isozyme band is identical to the of the EE satellite band of $Pgi\text{-}2^{100}$ homozygotes, but is characterized by a faint anodal smear. In populations in which the $Pgi\text{-}2^{147}$ is present at sufficiently high frequencies, $Pgi\text{-}2^{147}$ homozygotes occur, which lack both the B^1B^1 and B^1B^2 isozyme bands (Fig. 3). The absence of the A^1B^1 isozyme band in $Pgi\text{-}2^{147}$ homozygotes is conspicuous.

The $Pgi\text{-}2^{147}$ allele is present in both Little Campbell

leiurus and trachurus populations at a low frequency, and results of the lab crosses support the interpretation of variability in the PGI-2 region as that due to two alleles segregating at a single autosomal locus (Table 5). In most crosses, both parents were homozygous for $Pgi\text{-}2^{100}$, and therefore possessed the B^1B^1 isozyme, and all offspring of these crosses also possessed only the B^1B^1 isozyme. In two of the crosses involving trachurus, and one involving leiurus, one parent was a $Pgi\text{-}2^{100}/Pgi\text{-}2^{147}$ heterozygote and displayed all three of the B^1B^1, B^1B^2 and B^2B^2 isozyme bands. One-half of the progeny of each of these crosses were homozygous for the $Pgi\text{-}2^{100}$ allele and possessed only the B^1B^1 isozyme, while the other half were heterozygous for $Pgi\text{-}2^{100}/Pgi\text{-}2^{147}$ and possessed the B^1B^1, B^1B^2 and B^2B^2 isozymes.

The isozyme band A^1A^1 representing the homodimeric association of peptides coded for by the $Pgi\text{-}1^{100}$ allele at the Pgi-1 locus was not well resolved under the electrophoretic conditions of this study. The isozyme band was wide and tended to smear towards the anode (Fig. 3). This made the variability difficult to score; however, some populations did possess a variant allele $Pgi\text{-}1^{105}$ that coded for a peptide which in homodimeric form (A^2A^2) migrated slightly faster than A^1A^1.

The closely spaced A^1A^1, A^1A^2, A^2A^2 bands of a $Pgi\text{-}1^{100}/Pgi\text{-}1^{105}$ heterozygote often are difficult to distinguish from the wide smear of the A^1A^1 band in a $Pgi\text{-}1^{100}$ homozygote. The A^2B^1 hybrid isozyme band of heterozygotes is also difficult to detect because it is similar to the EE band of homozygotes. However, like the A^1B^2 isozyme band it tended to extend anodally

Table 5. Inheritance of Pgi-2 alleles in Little Campbell River trachurus and leiurus sticklebacks. In both sets of crosses the faster allele (F) is Pgi-2^{107} and the slower allele (S) is Pgi-2^{100}. Parental genotypes in brackets were inferred from progeny genctypic ratios.

Parental Genotypes	No. of Crosses	Progeny FF	Progeny FS	Progeny SS	Parental Genotypes	No. Of Crosses	Progeny FF	Progeny FS	Progeny SS
Trachurus									
♂ SS ♀ SS	10	0	0	167	♂ FS ♀ SS	1	0	6	4
♂ (SS) ♀ (SS)	4	0	0	56	♂ SS ♀ FS	1	0	4	6
Leiurus									
♂ SS ♀ SS	5	0	0	281					

and could usually be distinguished from the EE band on this basis. Nevertheless, the frequency of the Pgi-1^{105} allele, never high to begin with, may be underestimated in some populations because of scoring difficulties. The allele was not detected in either of the Little Campbell populations (parents or progeny) and therefore its inheritance could not be studied. However, the occurrence of putative Pgi-1^{105} homozygotes in a few populations supports the genetic interpretation of this variability. These individuals possess fast-migrating A^2A^2 and A^2B^1 isozyme bands and, as expected, lack the A^1A^1, A^1A^2 and A^1B^1 bands. Other alleles occur at the Pgi-1 locus in even lower frequencies. These alleles produce the characteristic 3-banded variability in the PGI-1 region in heterozygous condition, and a single band of altered mobility when homozygous.

Phosphoglucomutase

The electrophoretic pattern of Gasterosteus PGM is the most variable of all enzymes examined. Five anodal bands with distinct electrophoretic mobilities occur in varying frequencies among populations, and each fish possessed one or two of these bands (Fig. 4). This is consistent with the suggestion that PGM is a monomeric enzyme (Joshi et al. 1967) and each band is the product of a different allele of a single autosomal gene. Individuals displaying only one band are homozygous for one allele, 2-banded individuals are heterozygotes possessing two different alleles.

The most common allele, termed Pgm-^{100}, codes for the

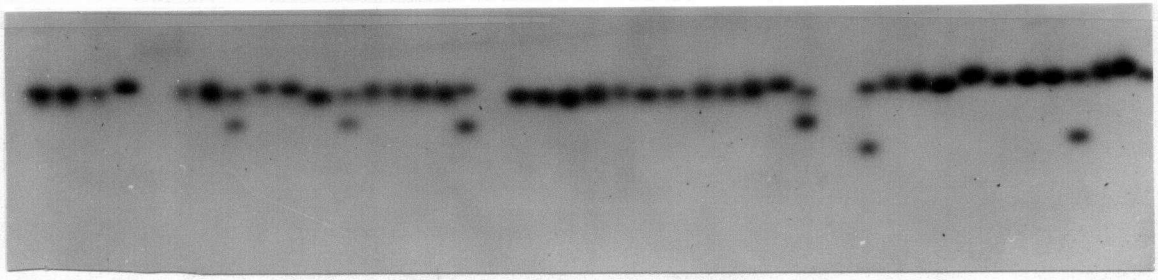
peptide which stains as band A (Fig. 4). Pgm^{103}, the next most common allele, codes for the peptide of band B, which migrates to a position slightly anodal to A. The alleles Pgm^{93} and Pgm^{90} code for the peptides staining as bands C and D respectively, and Pgm^{80} for the slow-migrating peptide of band E. After prolonged staining or incubation in elevated concentrations of glucose-1-phosphate, a second zone of PGM activity (PGM-1) can be detected in the gel region anodal to that containing the isozymes monitored in this study (PGM-2). Although this region was clearly polymorphic, low activity precluded its use.

The Little Campbell trachurus population possesses the Pgm^{100} allele (F) and, at a lower frequency, the Pgm^{90} allele (S) (Table 6). Crosses involving all possible parental genotypic combinations of homo- and heterozygotes confirmed that the isozyme bands observed are indeed under the control of two codominant alleles at a single autosomal locus.

The Little Campbell leiurus population contains the Pgm^{103} (F) and the Pgm^{100} (S) alleles (Table 6). Homo- and heterozygotes for these alleles were employed as parents in the lab crosses, and in each case the offspring displayed genotypic ratios in accordance with Mendelian predictions based on the premise that these are two alleles of a single gene.

Inheritance patterns of the Pgm^{93} and Pgm^{80} alleles, not present in the Little Campbell populations, could not be examined. However, as with the confirmed alleles, their presence in a homozygous or heterozygous state results in the production of either one or two isozyme bands. Further support for the proposed genetic interpretation of the observed variability is

Figure 4. The PGM isozyme banding patterns of threespine sticklebacks. The Pgm^{100} allele codes for the peptide of band A, Pgm^{103} for that of band B, Pgm^{93} for that of band C, Pgm^{90} for that of band D and Pgm^{80} for that of band E. Homozygotes (nos. 3 and 4) for any allele display the single band formed by the peptide product of that allele. Heterozygotes (nos. 1, 2, 5 and 6) display the two bands formed by the respective peptide products of their two alleles.



PGM

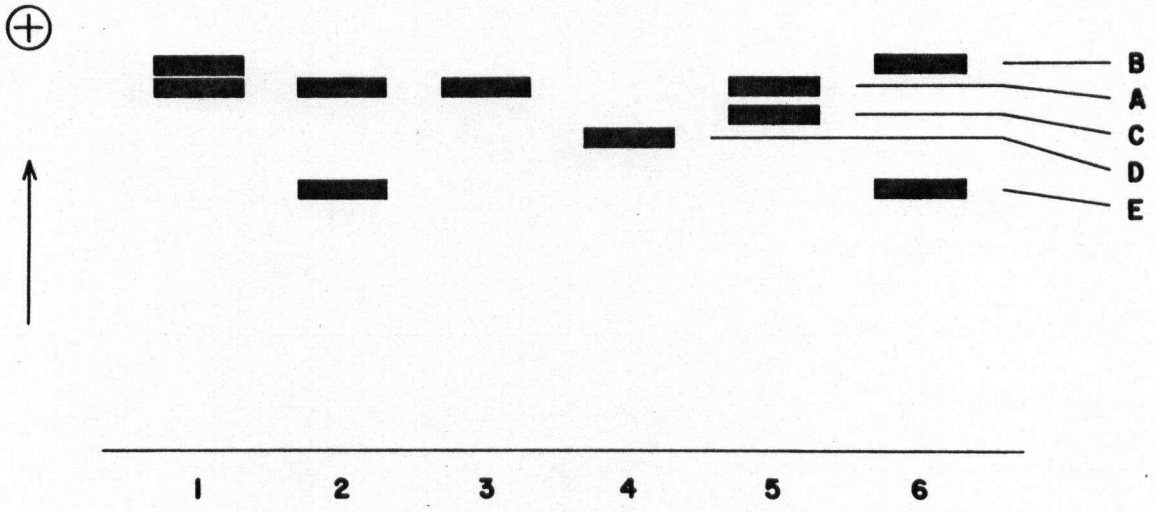


Table 6. Inheritance of Pgm alleles in Little Campbell River trachurus and leiurus sticklebacks. In the trachurus crosses, the faster allele (F) is Pgm^{100} and the slower allele (S) is Pgm^{90}. In the leiurus crosses, F is Pgm^{103} and S is Pgm^{100}. Parental genotypes in brackets were inferred from progeny genotypic ratios.

Parental Genotypes		Progeny			Parental Genotypes		Progeny		
Male	Female	FF	FS	SS	Male	Female	FF	FS	SS
Trachurus									
(1) FF	(1) FF	16	0	0	(8) FS	(9) SS	0	5	6
(2) FS	(2) FS	15	30	17	(8) FS	(10) FF	5	5	0
(3) FS	(3) FF	4	6	0	(9) SS	(9) SS	0	0	6
(4) FF	(4) FF	14	0	0	(9) SS	(10) FF	0	11	0
(5) (FF)	(5) (FF)	16	0	0	(10) FF	(11) FF	10	0	0
(5) (FF)	(6) (FS)	11	12	0	(10) FF	(12) FS	4	3	0
(6) FF	(7) FS	4	6	0	(11) FF	(12) FS	5	5	0
(7) FS	(8) FF	10	10	0	(12) (FS)	(13) FF	4	6	0
Leiurus									
(13) FS	(15) FS	16	23	17	(15) FF	(18) FS	20	19	0
(13) FS	(16) FF	29	18	0	(16) FS	(19) SS	0	27	31
(14) FF	(17) FF	77	0	0					

that allele and genotype frequencies calculated from the isozyme banding patterns indicate that populations are in Hardy-Weinberg equilibrium. Other rare alleles are present in some populations.

The variability observed at the Pgm locus of Gasterosteus closely resembles, both in quantity and in nature, that of other vertebrates. PGM is characteristically heterogeneous within species, and single-banded homozygous and double-banded heterozygous phenotypes are typical (see, for example, Roberts et al., 1969, Lush 1969, Utter and Hodgins 1970).

Lactate Dehydrogenase

Most fish species possess three structural loci coding for polypeptide units of lactate dehydrogenase that are commonly referred to as Ldh-A, Ldh-B and Ldh-C (Markert et al., 1975). In teleosts, expression of the A and B loci, although variable between species, is generally widespread throughout body tissues, while expression of the C locus is usually confined to neural tissue such as eye and brain, or, in a few species, the liver. LDH is a tetramer and in vertebrates other than fish the peptides coded for by the A and B genes associate randomly to produce binomial proportions of the five isozymes 4A, 3A1B, 2A2B, 1A3B, 4B. In many fish species, however, the association of peptides coded for by the two genes is restricted, and not all five isozymes are formed. Some species possess only the two homotetrameric isozymes 4A and 4B, others these two homotetramers plus the hybrid isozyme 2A2B. Moreover, in some advanced teleosts, expression of the B locus is restricted to a

few tissues, so that in many tissues only the 4A isozyme is present. This reduction of B gene activity occurs in some families of the order Perciformes, and all examined families in the orders Pleuronectiformes and Tetraodontiformes (Markert et al. 1975).

The Gasterosteus muscle LDH pattern consists of a single isozyme band. This is most likely the 4A isozyme, indicating that in Gasterosteus, as in the forementioned advanced teleosts, there is a reduction in B gene activity. In the present study, the stickleback muscle 4A isozyme displayed an extreme lack of electrophoretically-detectable variability. Only one fish, in the thousands examined, possessed a variant allele of Ldh-A. The isozyme pattern of this individual was 5-banded, indicating that stickleback LDH-A is indeed tetrameric and that the two A peptides produced by the normal and variant alleles associated to produce all possible tetrameric forms. The lack of LDH-A variability was apparent in the Little Campbell trachurus and leiurus crosses. The parents and progeny of all crosses possessed the 4A isozyme band only.

When young fish were ground up whole, or when adult eye tissue was employed, more LDH isozymes were detected. The eye pattern consists of five bands, much fainter than the muscle 4A band. The slowest-migrating of these bands is cathodal and the fastest anodal to the 4A band, indicating that these two bands represent the 4B and 4C homotetrameric enzymes. The intermediate bands represent the hybrid B-C isozymes. Variability in this banding pattern indicated that unlike the A gene, one or both of the B and C genes is polymorphic. Markert et al. (1975) in Fig.

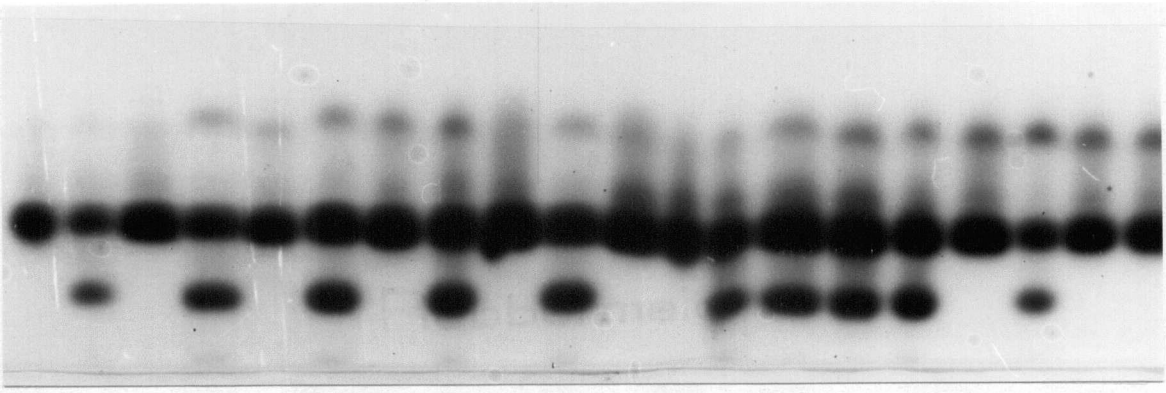
4, p. 111, indicate that the C gene is indeed expressed in the eye, rather than liver, of fish in the order Gasterosteiformes, but do not state which species were examined.

Isocitrate Dehydrogenase

Two forms of NADP-dependent IDH, coded for by separate structural genes, are present in vertebrate tissue (Henderson 1968, Quiroz-Gutierrez and Ohno 1970). As with MDH, one type functions primarily in the mitochondria, the other in the cell cytoplasm; and distribution of the two types varies among tissues. In mammals, mitochondrial IDH predominates in heart tissue, supernatant IDH in liver and kidney (Henderson 1968). Studies with fish reveal considerable variability in the tissue specificity of the two forms among species (Quiroz-Gutierrez and Ohno 1970, Engel et al. 1971, Reinitz 1977, Allendorf et al. 1975, Shaklee et al. 1974). However, one pattern that was common to all vertebrates examined in the above studies, with the exception of the surf smelt, was the greater anodal mobility of supernatant than of mitochondrial IDH. IDH is a dimer, and the characteristic 3-banded phenotype is displayed by supernatant IDH heterozygotes of mammaliam (Henderson 1968) and most fish (Quiroz-Gutierrez and Ohno 1970, Engel et al. 1971) species. However, Reinitz (1977) reported a 2-banded phenotype in rainbow trout heterozygous for supernatant IDH.

Gasterosteus muscle samples, run on Buffer II and stained for IDH, display one of two patterns (Fig. 5). The first consists of a single, darkly-staining isozyme band, A; the

Figure 5. Threespine stickleback IDH isozyme banding patterns. Females (nos. 1, 3 and 4) display the single A band, males (nos. 2 and 5) the A, B and C bands.



IDH

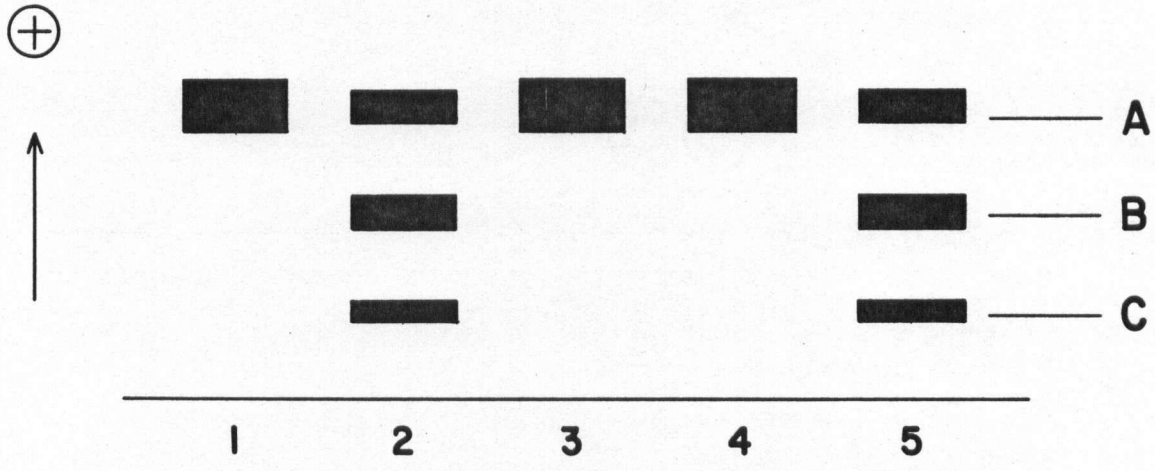


Table 7. Sex ratios in lab-reared broods of Little Campbell River trachurus and leiurus sticklebacks as determined by IDH electrophoretic patterns.

Cross	Progeny		Cross	Progeny	
	Males	Females		Males	Females
Trachurus					
1	25	18	8	3	7
2	3	7	9	0	6
3	5	5	10	3	8
4	6	10	11	6	4
5	7	3	12	4	3
6	5	15	13	6	4
7	4	7	14	3	7
	TOTAL:	80 Males	104 Females		
Leiurus					
15	32	27	18	21	21
16	24	20	19	33	25
17	30	47			
	TOTAL:	140 Males	140 Females		

second possesses, in addition to A, a second, more cathodal band B which migrates half as far from the origin as does A. In this second phenotype the A and B bands each stain approximately half as darkly as does the single A band of the first phenotype. In addition, the second phenotype possesses a very lightly staining band, C, which is located on the origin. Multiple bands trailing anodally from the A band are often present, and likely represent conformational isomers or chemically altered forms of the A isozyme. No other varibility was observed in IDH banding patterns among samples assayed in this study. Early in the survey the two patterns were recognized as sex-specific. Females display the single-banded and males the 3-banded phenotype.

Male and female Gasterosteus in breeding condition are easily differentiated, males possess bright red throats, females are gravid with eggs. Whenever such fish were present in the electrophoretic samples, sex was recorded and checked against IDH phenotype. Moreover, after muscle samples had been removed for electrophoresis from the non-breeding fish of several populations, they were dissected and the presence of ovaries or testes recorded. In no case was there a discrepancy between the sex of the fish as determined morphologically and as revealed by IDH phenotype. Thus the variability in IDH isozyme bands reflects a sexual dimorphism rather than allelic variation at an autosomal locus, as it was interpreted by Avise (1976). No fish examined in the present study displayed a variant phenotype, indicating that IDH is monomorphic in Gasterosteus (although Avise (1976) did report one sample that apparently possessed only the B band).

The inheritance studies using Little Campbell River fish revealed that sticklebacks as young as four weeks old display both patterns. Table 7 presents the sex ratios based on IDH phenotype of progeny of the crosses. Although the trachurus crosses show a nonsignificant excess of females ($X^2=3.13$, $P>0.05$), the larger sample sizes of the leiurus crosses reveal a 1:1 ratio.

Whether this dimorphism is the result of a sex-specific difference at an IDH locus itself, or at some other locus affecting the enzyme product of the IDH locus has not been determined. However, successful hormonal treatments employed to alter the sexual development of sticklebacks (i.e. cause genotypic females to develop into phenotypic males and vice verse) apparently did not affect the banding patterns of individuals (unpub. data). This indicates that the B and C bands of males are not brought about by post-translational enzyme modifications mediated by the male physiological environment as was the case with a sex-influenced electrophoretic variant of glucose-6-phosphate dehydrogenase in Drosophila (Komma 1968).

Several possible explanations remain. The B and C band isozymes may be the products of an autosomal sex-limited gene that is expressed only in genotypic males (presumably XY in Gasterosteus). The phenotypic feminization (by hormonal treatment) of genotypic males does not prevent expression of this gene, just as the phenotypic masculinization of genotypic females does not induce it. Such a gene has been described in Drosophila by Fukunaga et al. (1975) and Tanaka et al. (1976). Maleless (mle) is a recessive gene located on the autosomal

second chromosome. When homozygous, this gene is lethal in males but not in females. The use of sex-transforming genes to alter the phenotypic sex of both genotypic male and female Drosophila did not alter the specificity of lethality. Individuals with one X chromosome, whether phenotypically male or female, died; while individuals with two X chromosomes, whether phenotypically male or female and with or without an extra Y chromosome, survived. In a similar fashion, the B and C isozymes in Gasterosteus may be the products of an autosomal gene that is expressed in genotypic males, even when these fish have undergone hormonally-induced feminization. Alternatively, the gene of the B and C isozymes may be located on a male-specific chromosome, possibly the first Y-linked electrophoretic gene to be described for any organism.

There is no obvious reason for a sexual dimorphism in Gasterosteus muscle tissue IDH isozymes. Although polymorphisms for supernatant IDH have been reported for a number of fish species, no sex-linked or sex-limited effects have been noted. The cellular origin (supernatant or mitochondrial) of the stickleback IDH isozymes of this study was not determined, nor were IDH patterns in tissues other than muscle examined. A possibility worth examining is that the A band constitutes supernatant IDH, while the cathodal B and C bands of the male are mitochondrial IDH which has a different pattern of tissue expression in the female.

SECTION II. GENETIC RELATIONSHIPS IN *GASTEROSTEUS ACULEATUS*

Results

My analysis of genetic variability in 79 natural populations of *G. aculeatus* is presented in two parts. Since an evaluation of the significance of differences in allele frequencies and distributions among populations is possible only by comparison with the amount of intrapopulation variability, the analysis of electrophoretic variability among populations is preceded by an examination of polymorphism, heterozygosity and allele distributuions within populations.

Genetic Variability within Populations

For all populations, the sample size, allele frequencies, observed and expected numbers of heterozygotes, and level of heterozygosity for each of the six polymorphic loci are presented in Appendices II to VII. Table 8 contains a summary of the intrapopulation variability.

Polymorphism

Within each population, a locus was designated polymorphic if the frequency of the most common allele was less than, or equal to, 0.99. LDH and IDH were monomorphic in all populations. LDH possessed a single electrophoretically distinguishable allele and IDH displayed an invariant but sexually dimorphic

Table 8. Summary of intrapopulation genetic variability in Gasterosteus aculeatus.

Population	Average No. Genes Sampled (± S.E.)	Polymorphic Loci	Heterozygotes[1] Obs (Exp)	Average Heterozygosity (± S.E.)
Marine				
ENGLR	59.8 ± 0.7	4	17 (17)	0.082 ± 0.043
HORSB[2]	62.0 ± 23.9	2	12 (11)	0.044 ± 0.026
WITLG	63.5 ± 12.4	5	23 (21)	0.088 ± 0.047
LC 19	95.3 ± 2.0	4	58 (53)	0.117 ± 0.065
COWIB	54.0 ± 0.0	3	18 (16)	0.073 ± 0.051
MDERA[2]	41.3 ± 19.0	4	20 (19)	0.107 ± 0.050
SOOKP	64.8 ± 1.4	5	24 (23)	0.093 ± 0.037
KOKSR	134.5 ± 3.5	4	53 (55)	0.101 ± 0.045
SLMNC	106.0 ± 0.0	5	48 (58)	0.147 ± 0.066
GRAPI	82.0 ± 0.0	6	38 (41)	0.130 ± 0.051
BAMFS	67.8 ± 0.7	6	40 (44)	0.170 ± 0.047
SARIE	87.8 ± 0.7	5	47 (49)	0.139 ± 0.044
HAINL	46.0 ± 0.0	4	19 (22)	0.122 ± 0.052
CONGR	78.3 ± 7.2	6	40 (38)	0.127 ± 0.031
SANMA	88.0 ± 12.3	5	46 (46)	0.124 ± 0.044
CHEHW	32.0 ± 0.0	5	18 (18)	0.137 ± 0.051
Large Lake				
COWIL	74.0 ± 0.0	4	19 (22)	0.082 ± 0.050
SARIL	114.0 ± 0.0	5	88 (97)	0.213 ± 0.068
SAKIN	22.0 ± 0.0	5	14 (10)	0.127 ± 0.055
SPROT	275.5 ± 0.8	4	167 (178)	0.162 ± 0.075
MCRTL	509.8 ± 34.0	4	300 (319)	0.153 ± 0.069
GRCEN	105.5 ± 1.3	5	60 (69)	0.202 ± 0.077
STELL	37.0 ± 1.0	5	22 (21)	0.148 ± 0.053
ROBTL	42.0 ± 0.0	2	13 (11)	0.068 ± 0.058
Small Lake				
CHEML	162.8 ± 3.5	3	53 (51)	0.079 ± 0.043
FULLL	70.3 ± 4.1	4	73 (67)	0.247 ± 0.089
MARNL	149.8 ± 0.7	3	59 (57)	0.095 ± 0.048
LKERR	92.0 ± 0.0	4	47 (42)	0.115 ± 0.060
HOTEL	228.0 ± 0.0	5	123 (128)	0.142 ± 0.076
KLEIN	90.0 ± 0.0	4	53 (49)	0.140 ± 0.082
PAQLK	82.0 ± 0.0	2	19 (21)	0.065 ± 0.054
TROUT	100.0 ± 0.0	4	63 (57)	0.144 ± 0.066
GARBY	28.0 ± 0.0	2	13 (10)	0.088 ± 0.073
BLACK	509.8 ± 3.9	3	265 (288)	0.144 ± 0.084
PATER	213.5 ± 0.9	2	80 (77)	0.092 ± 0.075
DEVIL	169.3 ± 1.0	1	46 (42)	0.062 ± 0.058
SUMNL	131.3 ± 1.4	1	43 (46)	0.087 ± 0.077
MUDLK	118.0 ± 1.3	1	23 (23)	0.050 ± 0.046
LOWRL	94.0 ± 0.0	3	22 (24)	0.058 ± 0.048
MCOYL	259.5 ± 0.9	4	41 (43)	0.041 ± 0.018
CECIL	205.8 ± 0.7	3	118 (118)	0.143 ± 0.067
MORGL	161.3 ± 1.4	2	36 (34)	0.053 ± 0.040

ORMND	124.0 ± 0.0	2	21 (20) 0.041 ± 0.029
FARWL	277.5 ± 0.9	4	182 (176) 0.159 ± 0.085
CEDAR	216.0 ± 1.0	2	105 (100) 0.118 ± 0.074
PAXTB	220.3 ± 13.0	5	76 (75) 0.086 ± 0.047
PAXTL	87.3 ± 2.0	6	67 (68) 0.201 ± 0.066
ENOSB	230.5 ± 41.1	3	63 (64) 0.096 ± 0.059
ENOSL	307.0 ± 2.0	4	143 (149) 0.121 ± 0.061
GOOSE	131.8 ± 2.7	4	79 (78) 0.151 ± 0.067
HECHL	70.3 ± 6.3	4	46 (49) 0.186 ± 0.085
CRANL	95.3 ± 1.0	2	51 (47) 0.126 ± 0.077
Low-lying Stream or Swamp			
SKOOS[3]	61.0 ± 23.1	3	9 (10) 0.042 ± 0.025
SHIPC	54.0 ± 0.0	4	16 (19) 0.088 ± 0.042
LC 18	173.8 ± 0.7	4	113 (112) 0.161 ± 0.072
GIFFS[4]	22.8 ± 8.6	3	6 (6) 0.061 ± 0.025
TEXAS	49.8 ± 4.5	5	31 (29) 0.157 ± 0.063
PATBS	98.0 ± 0.0	4	79 (86) 0.221 ± 0.091
EBEYI	136.0 ± 0.0	4	91 (101) 0.188 ± 0.075
SKAGI	74.0 ± 0.0	6	44 (47) 0.190 ± 0.076
MOUSE	71.5 ± 1.3	6	50 (49) 0.175 ± 0.068
SMUDL	148.3 ± 20.5	3	111 (112) 0.180 ± 0.082
NATHN[5]	76.0 ± 26.6	4	61 (60) 0.251 ± 0.098
CLQTZ	94.0 ± 0.0	4	67 (65) 0.173 ± 0.066
MCRPD	199.0 ± 1.4	5	134 (128) 0.161 ± 0.066
SARIJ	76.3 ± 3.7	4	43 (54) 0.184 ± 0.072
TYNEP	625.2 ± 27.3	4	340 (373) 0.151 ± 0.068
HOQUB	66.0 ± 0.0	4	19 (20) 0.075 ± 0.038
OTTER	233.3 ± 1.4	3	86 (87) 0.106 ± 0.056
FARBL	343.8 ± 0.7	4	207 (211) 0.154 ± 0.081
Isolated Stream or Swamp			
KEOGH	90.0 ± 0.0	1	27 (29) 0.081 ± 0.076
FARSW	149.8 ± 0.7	3	64 (71) 0.122 ± 0.063
SPRPD	145.8 ± 0.7	3	71 (78) 0.059 ± 0.046
SLZER	103.0 ± 32.9	3	64 (74) 0.152 ± 0.071
DRYRN	100.0 ± 0.0	1	23 (25) 0.062 ± 0.058
Mixed			
FRASR	208.0 ± 6.6	4	103 (108) 0.132 ± 0.068
LARDC	227.5 ± 32.7	5	165 (168) 0.194 ± 0.069
FULCR	126.0 ± 0.0	4	58 (60) 0.133 ± 0.058
LC 40	38.8 ± 14.7	5	43 (49) 0.144 ± 0.068

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[1] Excludes data for Pgi-1 locus.

[2] Missing data for Ck.

[3] Missing data for Mdh-3.

[4] Missing data for Pgm.

[5] Missing data for Pgi-2.

banding pattern. Of the polymorphic loci, all but Pgm were segregating for two major alleles. Pgm possessed five common alleles. Rare alleles were detected at all polymorphic loci except Mdh-3. The number of polymorphic loci per population ranged from 1 to 6 (12.5% to 75% of the 8 examined). The average level of polymorphism, calculated as the unweighted average over all populations (excluding mixed samples, samples from lakes containing two partially or completely distinct populations, and samples for which data for one or more loci were missing), was 3.76 (47%) (Table 8).

Heterozygosity

For a single genetic locus, heterozygosity is given by: $H = 1 - \sum_{i=1}^{n} p_i^2$, where n is the number of alleles at the locus, and p_i is the frequency of the i^{th} allele in the population. Heterozygosity provides a measure of intrapopulation variability that accounts not only for the absolute number of alleles of a gene (which is polymorphism), but also their frequency in the population. Quite simply, it is the proportion of individuals heterozygous at a locus, assuming that the population is in Hardy-Weinberg equilibrium.

In this study, levels of heterozygosity varied among loci and among populations. Appendices II to VII give the level of heterozygosity for each polymorphic locus in each population. Idh and Ldh were consistently monomorphic, so the value of heterozygosity for these loci was 0 for all populations. For polymorphic loci possessing two major alleles the maximum value

of heterozygosity is 0.5. For Pgm, with five alleles, the maximum value is 0.8. The presence of rare alleles at any locus increases the potential level of heterozygosity for that locus.

Heterozygosity for Ck ranged from 0.000 to 0.497 with an average value of 0.107 in the 78 populations examined (Appendix VI). For Pgi-1 the values ranged from 0.000 to 0.294 in 76 populations, with an average of 0.018. This value is undoubtedly low because of undetected Pgi-1 heterozygotes in many populations (Sect. I). For Pgi-2, the limiting values were 0.000 and 0.526, with an average value of 0.085 for 78 populations. Heterozygosity at the Mdh-1 locus varied between 0.000 and 0.500, averaging 0.101 over 79 populations. The range for Mdh-3 was from 0.000 to 0.500, with an average for 77 populations of 0.275. For Pgm, heterozygosity ranged from 0.000 to 0.665, with an average of 0.418 over 78 populations.

For each population, the average heterozygosity ($\bar{H}$) is the average of the individual heterozygosity values for all loci examined. The level of average heterozygosity ranged from 0.041 in McCoy (MCOYL) and Ormond (ORMND) lakes to 0.247 in Fuller Lake (FULLL) (Table 8). The unweighted average value of $\bar{H}$ over all populations (excluding mixed samples, samples from lakes containing two partially or completely distinct populations, and samples for which data for one or more loci were missing) was 0.124.

Hardy-Weinberg Equilibrium

For each polymorphic locus in each population the observed

number of heterozygotes and the number expected under Hardy-Weinberg equilibrium conditions are given in Appendices II to VII. The G-test, employing log likelihood ratios (Sokal and Rohlf 1969), was used to test the significance of the departure of observed genotypic frequencies from Hardy-Weinberg expectations for all loci (except Pgi-1) sufficiently polymorphic to generate three genotypic classes each containing at least 5 individuals within a population. In the case of Pgm, genotypic classes with expected values of less than 5 were lumped (homozygotes with homozygotes, heterozygotes with heterozygotes). Because allele frequencies tended to remain constant in populations sampled more than once (see below), data for these populations were lumped in order to enable a single test for each location (Appendices II to VII). With the exception of repeated samples obtained from the Serpentine River (TYNEP) which are not included in these results but discussed separately below, independent tests on multiple samples from a single location did not give different results. Genotypic ratios for Pgi-1 were not tested against Hardy-Weinberg predictions because of the low frequency of alleles other than the common $Pgi\text{-}1^{100}$ at this locus, and the difficulty of detecting them in heterozygous form (Sect. I). Omissions in the scoring of heterozygotes is the most likely explanation for the observed heterozygote deficiency for PGI-1 in many populations (Appendix IV).

For creatine kinase, an overall G-test on eight polymorphic populations indicated no departure of observed genotypic frequencies from Hardy-Weinberg equilibrium ($X^2=3.74$, d.f.=8,

P>0.90). When populations were tested independently, each with one degree of freedom, none deviated at the 5% level from predicted genotypic proportions. An overall G-test on seven populations polymorphic for Mdh-1 again indicated close agreement of observed with expected ratios (X^2=3.77, d.f.=7, P>0.80). Independent tests on each of the populations revealed no significant departures from expected values.

For the highly polymorphic Mdh-3 locus, the overall test on 32 populations was significant (X^2=50.7, d.f.=32, P<0.05), and when tested independently the genotypic ratios of three populations deviated from Hardy-Weinberg expectations at the 5% level (Appendix II). The three samples represent widely differing habitats and geographic locations. One (SARIL) was collected from Sarita Lake, a large lake on the west coast of Vancouver Isalnd, one (EBEYI) from a low-lying slough near the mouth of the Snohomish River in northwestern Washington and one (FARSW) from an isolated swamp to the north of, and draining into, Farewell Lake on eastern Vancouver Island. Three out of fifty is a slightly greater proportion than the 5% of samples expected to deviate from predicted values by chance alone, and all three populations show heterozygote deficiencies. This condition suggests the Wahlund effect (a shortage of heterozygotes resulting from the inclusion of individuals from more than one population with differing gene frequencies in a single sample). However, it could also result from nonrandom breeding among different Mdh-3 genotypes within a single population, or from disruptive, or variable, selective forces acting upon a single population.

Four populations polymorphic for Pgi-2 possessed genotypic ratios in full accordance with Hardy-Weinberg predictions ($X^2=3.35$, d.f.=4, $P>0.50$). When tested independently none of the populations deviated from expected values. For 37 populations polymorphic for the multi-allelic Pgm locus, the overall G-test showed no departure of observed from expected genotypic ratios ($X^2=70.07$, d.f.=64, $P>0.30$). Two populations in the Bear River system on Vancouver Island, one (MCRTL) from a large lake and one (FARWL) from a small lake, possessed genotypic frequencies significantly different from Hardy-Weinberg conditions when tested independently (Appendix VII). This number is no more than expected from sampling error. However, each of these locations was sampled twice and in neither case did the allelic or genotypic ratios vary between samples. The McCreight Lake samples possessed five alleles, and the deviation from equilibrium conditions resulted from a deficiency of heterozygotes possessing the two most common alleles. The Farewell Lake samples possessed four alleles, and there was a deficiency in two of the heterozygous classes and a surplus in two of the others.

Multiple Samples

Samples from the populations examined in this study were collected by a variety of methods, and at different times of the year. Gene frequency variability among populations may simply reflect differing gear selectivities, or seasonal fluctuations in gene frequencies. Several Vancouver Island lake populations

were sampled simultaneously by minnow traps and pole seine (or dip net) to determine if different gear types disproportionately sample different population segments which possess distinct gene frequencies. In general, minnow traps catch only the larger members of a stickleback population, whereas pole seines effectively sample the smaller size classes but not the faster-swimming and offshore-dwelling larger fish. Fish caught by the two methods in Blackwater Lake (BLACK) were subsampled, and their hypural length recorded before electrophoresis. The mean length of trap-caught sticklebacks was 5.07 cm (N=43, S.E.=1.4) and of pole-seined sticklebacks was 3.46 cm (N=40, S.E.=1.2).

For each population sampled by the two methods, a G value was obtained for the difference in allele frequency between the two samples for each polymorphic enzyme. The sum of the G values obtained for each enzyme was tested for goodness of fit to the X^2 distribution (Table 9). Differences in sex ratios (as determined by the IDH banding patterns) between trapped and seined samples were also tested. As Table 9 indicates, Mdh-1, Pgi-2 and Ck were each polymorphic in one population, Mdh-3 was polymorphic in three, and Pgm in all four of the populations sampled. There were no differences at the 5% significance level in allele frequencies between trap-caught and pole-seined samples for any of these enzymes. Frequencies of the sexually dimorphic banding pattern indicated that sex ratios were also similar among fish sampled by the two methods. Thus, there is no evidence that the fish caught by different methods constitute genetically distinct subgroups of a population.

However, if such variability does exist within populations,

differences in allele frequencies between age groups might be expected. Although sticklebacks are difficult to age with certainty, an attempt was made to test this hypothesis. Sticklebacks collected by pole seine from a roadside slough one mile east of Otter Point in Sooke, Vancouver Island (OTTER) comprised two distinct size classes, small (mean length=3.74 cm, N=66, S.E.=0.04) and large (mean length=4.92 cm, N=51, S.E.=0.10). The pronounced bimodality of the size distribution in this population suggests the presence of two distinct age classes, with the larger fish constituting the older age group. Three enzymes were polymorphic in the fish from this location, MDH-3, PGM and CK, but there were no differences in allele frequencies between the large and small fish (Table 9). Thus, to the extent that distinct size classes are indicative of distinct age classes, no differences in gene frequencies between age groups is indicated. If such differences do exist in the OTTER population, they are too small for present sample sizes to reveal.

To detect seasonal variation in allele frequencies within populations, several Vancouver Island lake populations in the Bear River and Somass River watersheds were sampled in both spring (May or June) and fall (Septempber) 1978. For a single location, multiple samples taken simultaneously by different methods in either the spring or the fall were combined for this comparison (previous results indicated no allele frequency differences between fish sampled by the various methods). The May and June samples were mainly adult fish caught inshore in breeding condition. Small, newly-hatched fish (young-of-year)

Table 9. Allele frequency variability in multiple samples from Gasterosteus populations. G values were calculated for allele distributions at individual loci among multiple samples. The critical chi-square value at the .05 level for each comparison is given in brackets.

	Mdh-1	Mdh-3	Pgi-2	Pgm	Ck	Idh
A. Vancouver Island populations: pole-seine vs. minnow trap						
No. populations:	1	3	1	4	1	4
Degrees freedom:	1	3	1	11	1	4
Obs. G value:	2.62	2.97	1.68	9.44	0.41	4.97
Crit. Chi-square:	(3.84)	(7.81)	(3.84)	(19.7)	(3.84)	(9.49)
B. Slough east of Otter Point: large vs. small fish						
Degrees freedom:	--	1	--	2	1	1
Obs. G value:	--	2.18	--	2.68	0.19	0.43
Crit. Chi-square:	--	(3.84)	--	(5.99)	(3.84)	(3.84)
C. Vancouver Island populations: May/June vs. September capture						
No. populations:	2	9	5	12	--	14
Degrees freedom:	2	9	5	26	--	14
Obs. G value:	2.46	9.26	2.10	32.0	--	47.5***
Crit. Chi-square:	(5.99)	(16.9)	(11.1)	(38.9)	--	(23.7)
D. Serpentine River pond: samples over time						
Degrees freedom:	--	4	4	8	4	4
Obs. G value:	--	40.3***	3.01	3.76	5.85	3.10
Crit. Chi-square:	--	(9.49)	(9.49)	(15.5)	(9.49)	(9.49)

E. Chemainus and Marion lakes: multiple samples

Degrees freedom:	3	--	3	3	--	3
Obs. G value:	4.95	--	1.32	0.74	--	3.60
Crit. Chi-square:	(7.81)	--	(7.81)	(7.81)	--	(7.81)

F. Enos Lake: 1977 vs. 1978

Benthic samples

Degrees freedom:	--	--	1	--	1	1
Obs. G value:	--	--	1.88	--	0.16	0.70
Crit. Chi-square:	--	--	(3.84)	--	(3.84)	(3.84)

Limnetic samples

Degrees freedom	--	1	1	2	1	1
Obs. G value:	--	6.26*	0.80	6.95*	0.07	91.2***
Crit. Chi-square:	--	(3.84)	(3.84)	(5.99)	(3.84)	(3.84)

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* $P < 0.05$

** $P < 0.001$

*** $P < 0.0001$

constituted a significant portion of the September samples, although adult fish were still present. Again, for each polymorphic enzyme in each population sampled, a G value was obtained for the difference in allele frequencies between the spring and fall samples. The sum of the G values obtained for each enzyme was tested for goodness of fit to the X^2 distribution. MDH-1 was polymorphic in two populations, PGI-2 in five, MDH-3 in nine and PGM in twelve populations. None of these enzymes show a significant difference (at the 5% level) in allele frequencies between the spring and fall samples (Table 9). A highly significant difference in the frequencies of the two IDH banding patterns ($P<0.001$) indicates that the sex ratios were not constant between sampling periods. When examined individually, each with one degree of freedom, 3 of the 14 populations reveal significant differences in sex ratio between spring and fall. One population had a larger proportion of females in June, two contained a greater number of females in the fall. However, as stated earlier and further supported by this example, allele frequencies at the polymorphic loci examined in this study do not differ between the sexes, so that variable sex ratios are not indicative of varying allele frequencies (except, perhaps, at any sex-determining loci that may exist).

To further examine the possibility of allele frequency variability over time, a single population in the Fraser Valley (TYNEP) was sampled several times over a 15 month period. The location consists of a small, highly eutrophic pond adjacent to the headwaters of the Serpentine River. The pond is flooded by

the river in the winter and isolated during the drier summer months. In the summer it shrinks to a fraction of its winter size. This pond possesses a dense stickleback population which must fluctuate in numbers with variations in pond size, and which probably experiences both immigration and emigration during flood periods. Thus the pond seemed a likely location to observe changes in allele frequencies over time, either in response to variable selective regimes, or to random events such as genetic drift or the differential immigration of genotypes.

Samples were pole seined from the pond in March 1978 (adults), June 1978 (separate collections of adults and young-of-year), August 1978 (combined collection of adults and young) and June 1979 (adults). There was an average of 44 fish per sample. Pgi-1 and Mdh-1 were monomorphic for the same allele in all samples. Pgi-2, Mdh-3, Ck and Pgm were polymorphic, and for each of these loci a G value was obtained for the allele frequency distributions in the five samples (Table 9). Only the value obtained for Mdh-3 was significant ($P<0.001$), indicating that allele frequencies at the other three loci remained constant over time. At the Mdh-3 locus, the Mdh-3^{100} allele was present at relatively high frequencies in the March 1978 and June 1978 (young-of-year) samples (0.26 and 0.23), and at much lower frequencies in the June 1978 (adult), August 1978 and June 1979 samples (0.09, 0.06 and 0.06). This apparently reflects a real change in allele frequencies over time, and although the cause cannot be ascertained it will be shown in later sections that Mdh-3 alleles may be subject to natural selection in Gasterosteus populations. The nonsignificant result for IDH

(Table 9) indicates that the sex ratio did not vary among samples.

Further evidence for the stability of gene frequencies over short time periods is provided by samples from Chemainus Lake (CHEML) on Vancouver Island and Marion (Jacobs) Lake (MARNL) in the U.B.C. Research Forest near Haney, B.C. Marion Lake, at an elevation of 1000 ft above sea level, contained no sticklebacks prior to an introduction in 1974 of 4000 Chemainus Lake Gasterosteus (J.D. McPhail, pers. comm.). Chemainus Lake was sampled with minnow traps in 1977 and 1978, and Marion Lake in 1977 and 1979. Mdh-3, Pgi-1 and Ck were monomorphic for the same allele in all four samples. Mdh-1, Pgi-2 and Pgm were polymorphic. G values calculated on the allele frequencies for each of these enzymes in the four samples are not significant at the 5% level (Table 9). This indicates that not only was there no change in allele frequencies within eiher of the lakes over a 1 or 2 year period, but that no differences in allele frequencies developed between the lakes, even after the two populations had been separated for several years. Thus, over this short time span there is no evidence for the action of genetic drift or natural selection upon the genetic variability detected by electrophoretic methods.

To this point, the sticklebacks inhabiting a single lake have been considered a single population, and the samples taken from a small portion of a lake representative of its entirity. The data presented so far support this idea to the extent that multiple samples taken over time, and in Cedar Lake (CEDAR) on Vancouver Island over collecting sites several hundred yards

apart, were homogeneous. However, intra-lake population subdivision remains a theoretical possibility, especially in large Vancouver Island lakes with their wide ranges of habitat diversity (i.e. Cowichan, Great Central and Sproat lakes). The difficulties in obtaining access to, and in sampling the primarily offshore-dwelling populations of these large lakes hindered attempts to discern intra-lake variability. The restricted samples obtained from each lake are therefore, by necessity, considered representative of the entire lake population(s).

Genetically distinct populations of G. aculeatus were, however, found to coexist in two small lakes, Paxton Lake on Texada Island and Enos Lake on Vancouver Island. Both lakes contain two morphologically distinct groups of sticklebacks, referred to as benthic and limnetic. In Paxton Lake, Larsen (1976) found that the two types differed in distribution, feeding behavior and aggressiveness, as well as morphology (the number of body plates, body size and shape, the number of gill rakers and presence or absence of pelvic girdle). The morphology and distribution of the two types in Enos Lake is similar to those of Paxton (J.D. McPhail, pers. comm.). In this study, the larger benthic sticklebacks (PAXTB and ENOSB) were caught by minnow traps in both lakes, and the smaller limnetic sticklebacks (PAXTL and ENOSL) by night-time seining. Paxton Lake was sampled in 1977, Enos in both 1977 and 1978.

Stability of the allele frequencies characterizing benthic and limnetic populations was examined by comparing the 1977 and 1978 samples of Enos Lake. For the benthic fish, allele

frequencies at the polymorphic Pgi-2 and Ck loci remained constant from year to year. For the limnetics, allele frequencies were constant between years at the Pgi-2 and Ck loci, but differed at the Mdh-3 ($P<0.025$) and Pgm ($P<0.05$) loci (Table 9). The high levels of average heterozygosity displayed by these populations (Table 8), uncharacteristic of populations inhabiting small lakes, seems associated with the presence of two distinct types of Gasterosteus in close coexistence.

Hiechhold (HECHL) and Cranby (CRANL) lakes on Texada Island, and Goose Lake (GOOSE) in the U.B.C. Research Forest possess sticklebacks displaying both benthic-like and limnetic-like phenotypes, as well as a distribution of phenotypes apparently intermediate to the two types (J.D. McPhail, pers. comm.). The absence of clearly bimodal distributions of phenotypes indicates that these populations may not be composed of two completely distinct genetic groups, and samples from these lakes are presented as samples from single populations. However, there is likely a degree of assortative mating beween like phenotypes in these lakes as well; and they also are characterized by high levels of average heterozygosity (Table 8).

Genetic Variability among Populations

In contrast to the general homogeneity of allele frequencies within populations of G. aculeatus, comparisons among populations reveal great genetic heterogeneity. G values calculated for the allele distributions at each of the polymorphic loci in all 79 populations are given in Table 10.

All are significant at the 5% level, indicating that there exist real differences in allele frequencies among populations. I examined patterns in the allele distributions, and in the amount of genetic variability characterizing different types of populations.

Comparison Of Freshwater And Marine Populations

To determine if the differences in life history between marine and freshwater sticklebacks are reflected in differing gene frequencies, a G-test was used to compare the allele frequencies at each polymorphic locus in marine and freshwater populations. Populations residing permanently in brackish or tidally-influenced water were excluded, but marine populations sampled while breeding in brackish estuarine regions were included. A total of 65 populations, 16 marine and 49 freshwater, were examined. Allele frequencies at two loci differed between the two, Pgm ($P<0.005$) and Mdh-3 ($P<0.001$). At the Ck, Mdh-1 and Pgi-2 loci, there were no differences in allele frequencies between marine and freshwater populations (Table 10).

Nei's (1972, 1975) measure of genetic distance (D) makes possible an evaluation of the differences between freshwater and marine populations based on the information provided by allele frequencies at all loci. The minimum genetic distance between two populations, X and Y, is calculated as the mean of $D = (x_i - y_i)^2 / 2$ over all loci, where x_i is the frequency of the i^{th} allele in X and y_i is the frequency of the i^{th} allele in

Table 10. Allele frequency variability among *G. aculeatus* populations. Values of average frequencies are not weighted by sample size. Critical chi-square and F values at .05 level are in brackets.

Populations	Average Allele Frequencies						
	Mdh-1 (100)	Mdh-3 (100)	Pgi-2 (100)	Ck (85)	Pgm (100)	Pgm (90)	Pgm (103)
Marine	0.953	0.147	0.941	0.955	0.759	0.183	0.046
All Freshwater	0.923	0.635	0.944	0.879	0.554	0.173	0.211
Large Lakes	0.870	0.552	0.945	0.965	0.725	0.155	0.107
Small Lakes	0.970	0.653	0.934	0.891	0.501	0.183	0.242
Low-lying Streams	0.897	0.550	0.959	0.780	0.603	0.155	0.201
Isolated Streams	0.860	0.563	1.000	1.000	0.674	0.191	0.087
Overall Chi-square:	1423.1 (99.6) ***	5310.8 (97.4) ***	867.8 (97.4) ***	4058.7 (98.5) ***		8947.5 (349.6) ***	
Marine-Freshwater F Value:	0.44 (3.99)	12.61 (4.00) **	0.46 (4.00)	1.32 (4.00)		3.95 (2.41) *	
Among Habitat F Value:	2.43 (2.52)	4.81 (2.52) *	1.76 (2.52)	9.69 (2.52) ***		2.14 (2.52)	

* $P < 0.05$

** $P < 0.001$

*** $P < 0.0001$

Y. Genetic distances between all pairs of 16 marine, all pairs of 41 freshwater and all pairs of one each of these marine and freshwater populations were calculated. Populations from lakes containing two completely or partially distinct populations (Paxton, Enos, Goose, Hiechhold and Cranby) were excluded from this analysis, as were any populations lacking electrophoretic data for one or more loci.

The average genetic distance between marine populations was 0.0055 ± 0.0011 (range from 0.0005 to 0.0239, 120 pairwise comparisons), between freshwater populations was 0.0632 ± 0.0072 (range from 0.0001 to 0.2345, 820 comparisons) and between marine and freshwater populations was 0.0608 ± 0.0126 (range from 0.0010 to 0.2202, 656 comparisons). The low genetic distance between marine populations indicates a great similarity in allele distributions among them, while the larger distance between freshwater populations indicates allelic differentiation. The average genetic distance between marine and freshwater populations was similar to that between pairs of freshwater populations, indicating that, on average, the genetic differences between marine and freshwater stickleback populations were no greater than between two freshwater populations. However, the average marine-freshwater genetic distance was much greater than that between pairs of marine populations.

Geographic Variability In Allele Frequencies

To test for clinal variation in allele frequencies, simple

regressions of frequencies of the most common alleles at polymorphic loci were performed, employing longitude and latitude as the independent variables. Frequencies of $Mdh\text{-}1^{100}$, $Mdh\text{-}3^{100}$, Ck^{85}, $Pgi\text{-}2^{100}$, Pgm^{100}, Pgm^{103} and Pgm^{90} were subjected to arcsin square root transformation before use. Since at some loci significant allele frequency differences exist between sticklebacks in marine and freshwater habitats, separate analyses were performed on marine and freshwater populations. Mixed samples and samples from lakes containing two populations were excluded. A total of 16 marine and 51 freshwater populations were employed.

Among freshwater populations, frequencies of the Pgm^{90} ($P=0.05$, $R^2=0.07$, $N=50$) and Ck^{85} ($P=0.03$, $R^2=0.08$, $N=51$) alleles were significantly correlated with latitude. The frequency of Pgm^{90} increased and Ck^{85} decreased in a northerly direction. Freshwater frequencies of the remaining alleles were not correlated with latitude. Similarly, only the frequency of Ck^{85} ($P=0.05$, $R^2=0.07$, $N=51$) and Pgm^{90} ($P=0.01$, $R^2=0.12$, $N=50$) were correlated with longitude in freshwater. Their frequencies increased and decreased, respectively, in a westerly direction.

None of these correlations were highly significant and do not provide strong evidence for clinal variation in freshwater allele frequencies. Only two out of 14 regressions had probabilities of less than 0.05, a proportion which may be attributable to chance alone. In the next section, the frequency of Ck^{85} is shown to be significantly higher in low-lying streams than in other freshwater habitats. The latitudinal cline in Ck^{85} frequency probably reflects the fact that all but one of the

southern (i.e. Washington) populations that were sampled came from low-lying streams. If samples from Washington lakes were included, that apparent north-south cline in Ck^{85} frequency would probably disappear. On the other hand, it is possible that the northerly and easterly increase in Pgm^{90} frequency results from selection by an environmental variable that displays a similar cline, but very little of the allele frequency variability (7% and 12%) is explained by these geographic trends.

Among marine populations, none of the alleles examined were significantly correlated with latitude. Since the study included only one marine sample from Washington, the latitudinal range was obviously limited and perhaps insufficient to allow detection of clinal variation. However, frequencies of Pgm^{103} ($P=0.0002$, $R^2=0.64$, $N=16$), Ck^{85} ($P=0.0003$, $R^2=0.66$, $N=15$) and $Pgi\text{-}2^{100}$ ($P=0.06$, $R^2=0.23$, $N=16$) were correlated with longitude. Frequencies of Ck^{85} and $Pgi\text{-}2^{100}$ (both common alleles) decreased in a westerly direction, while the frequency of Pgm^{103} (a less common allele) increased.

All of the marine samples in the study except the one from Washington State (CHEHW) were collected either from the west coast of Vancouver Island (SLMNC, GRAPI, BAMFS, SARIE, HAINL, CONGR, SANMA) or from the more easterly Strait of Georgia, separating Vancouver Island from the B.C. mainland (ENGLR, HORSB, WITLG, LC 19, COWIB, MDERA, SOOKP, KOKSR). To determine if the significant regressions of Pgm^{103}, Ck^{85} and $Pgi\text{-}2^{100}$ frequencies on longitude were the result of genetic differentiation between these two regions, the G-test was used

to compare allele frequencies at all polymorphic loci between the pooled samples of each region. As expected, significant allele frequency differences were demonstrated for the Pgm ($G=56.08$, $P<0.0001$), Pgi-2 ($G=11.40$, $P<0.001$) and Ck ($G=58.48$, $P=0.0001$) loci. No difference in the allele frequencies of the two regions was apparent at the Mdh-1 ($G=0.97$, $P>0.30$) or Mdh-3 ($G=1.67$, $P>0.15$) loci.

Comparisons Of Allele Frequencies Among Habitats

The relative genetic homogeneity found among marine populations that share a somewhat uniform oceanic habitat contrasts sharply with the genetic heterogeneity found among populations occupying diverse freshwater habitats. This contrast suggests the possibility of gene-environment associations. To examine this possibility the populations sampled were classified into five categories according to habitat type; marine; large lake (>1 km^2); small lake (<1 km^2); low-lying stream or swamp; and isolated stream or swamp (as in Table 9). Low-lying streams and swamps are those at elevations less than 100 m above sea level, and those at elevations greater than 100 m in drainage systems with lakes or streams known to contain sticklebacks at higher elevations. Isolated streams and swamps are those at elevations greater than 100 m with no direct or indirect inflow from bodies of water known to contain sticklebacks. This apparently simplistic classification of habitats actually encompasses a large number of environmental variables including water flow, temperature regimes, vegetation types and number and

type of predators. All samples obviously composed of sticklebacks from more than one population (Mixed), including samples from the small lakes possessing benthic and limnetic fish, were excluded from the analysis.

For each of the polymorphic enzymes a one-way analysis of variance was performed on the frequency of the most common allele (arcsin square root transformed) in each population. The results are presented in Table 10. The frequency of the Ck^{85} allele was highly heterogeneous among types of populations ($P<0.00001$). A Duncan's multiple range test (Steel and Torrie 1960) indicated that the frequency of Ck^{85} was significantly lower in low-lying stream or swamp populations than in all other types of populations. The ANOVA results for the Mdh-1 allele approached significance ($P=0.06$). The frequency of this allele was lower in populations from large lakes and low-lying streams than from the ocean, small lakes and isolated streams. The $Pgi\text{-}2^{100}$ allele was not significantly heterogeneous ($P=0.15$) among habitats. The $Mdh\text{-}3^{100}$ allele frequency was heterogeneous ($P=0.002$). As shown previously, a Duncan's range test indicated that its frequency was lower in marine than in all freshwater populations; there was no significant heterogeneity among the different types of freshwater habitats. The ANOVA for the frequency of Pgm^{100} approached significance ($P=0.09$); the frequency of this allele was lowest in small lake populations and highest in marine populations.

Comparisons Of Genetic Variability Among Habitats

The frequencies of the most common alleles at a number of loci were lowest in populations inhabiting large lakes and low-lying streams and swamps. This indicates that either the number or the frequency, or both, of less common alleles in these populations is higher than in those occupying small lakes and isolated streams or swamps. To examine this possibility an analysis of variance using the same habitat classification scheme was performed on the number of polymorphic loci per population. Populations with data missing for one or more loci were excluded. The highly significant result ($P<0.00001$) confirms the presence of heterogeneity in the amount of polymorphism among different types of populations. A Duncan's multiple range test showed that the number of polymorphic loci was significantly lower in populations inhabiting small lakes and isolated streams than in other populations. Marine populations possessed the highest average number of polymorphic loci (4.8), followed by large lakes and low-lying streams (4.3), and finally by small lakes (2.8) amd isolated streams (2.2).

An analysis of variance performed on the levels of average heterozygosity (values arcsin square root transformed) in populations occupying the five habitat categories was also significant ($P=0.001$). Average heterozygosity was higher in large lakes and low-lying streams than in other populations. The average value of H was 0.158 in low-lying streams and swamps, 0.144 in large lakes, 0.118 in marine populations, 0.103 in small lakes and 0.095 in isolated streams and swamps.

These results indicate that populations inhabiting small lakes or isolated streams possessed little genetic variability;

they were characterized both by low polymorphism and low heterozygosity. Marine populations, the most polymorphic of all examined, possessed the greatest numbers of variant alleles. However, they displayed an intermediate level of heterozygosity indicating that these variant alleles did not occur at high frequencies. The sticklebacks of large lake and low-lying stream populations displayed fewer alleles than marine populations (i.e. they had lower polymorphism) but the variant alleles they did possess were present at higher frequencies (i.e. they had higher heterozygosity).

Comparisons Within And Between Drainage Systems

Extending the study of patterns of genetic variability, I questioned if the allele frequencies in different types of habitats within a drainage system were more similar to each other than were allele frequencies in the same type of habitat in different drainage systems. Two watersheds were sampled extensively for this purpose, the Somass River system draining eastward and the Bear River system draining westward on Vancouver Island. Eight locations in the Somass and eleven in the Bear, representing a variety of habitats, were sampled, many of them in both the spring and fall of 1978. Habitat classifications used in the analysis were large lake (>1 km^2), small lake (<1 km^2) and stream or swamp. A completely nested analysis of variance was performed on the allele distributions at each of the polymorphic loci, Pgi-2, Pgm, Mdh-1 and Mdh-3. The levels of the analysis were drainage system, habitat type

within drainage system, individual location within habitat type and, finally, multiple samples from within a single location.

The results of the analysis are given in Table 11. For every enzyme, the heterogeneity in allele distributions between locations was highly significant ($P<0.001$). Allele frequencies did not differ significantly between river systems and varied between habitat types only in the case of Mdh-1 ($P<0.05$). This analysis indicates that the large amount of genetic variability observed among freshwater stickleback populations is not due to gene frequency differences between drainage systems nor between type of habitat occupied. Neither of these factors accounted for the great variability among locations. However, the previous analysis involving many more populations showed that the allele frequencies of some loci were related to habitat type, and the negative results of the present analysis may be at least partly due to small sample sizes. Examination of the components of variance (Appendix VIII) indicates that for Mdh-3 34% and for Pgm^{93} 30% of the variance in gene frequencies was due to differences between the two drainage systems. The significance of these apparent differences could be tested by extending the analysis to include a greater number of watersheds.

To examine differences in allele frequencies within and between drainage systems using the information from all loci combined, the genetic distances between all pairwise combinations of populations within each of the Bear and Somass systems, and all pairwise combinations of populations between the two systems, were calculated. The average genetic distance between populations within both the Bear ($N=55$) and Somass

Table 11. Allele frequency variability within and between the Bear and Somass watersheds. A completely nested analysis of variance was performed on the arcsin transformed frequencies of the most common allele(s) at each polymorphic locus.

Locus	Source of Variability	Sum of Squares	Degrees Freedom	Mean Square	F Ratio	Probability
Mdh-1	Between systems	0.008	1	0.008	0.08	0.85
	Among habitats	0.290	4	0.072	4.00	0.03
	Among locations	0.246	13	0.019	9.51	0.00
	Residual	0.024	11	0.002		
Mdh-3	Between systems	1.307	1	1.307	3.99	0.21
	Among habitats	1.096	4	0.274	1.50	0.25
	Among locations	2.452	13	0.189	63.05	0.00
	Residual	0.033	11	0.003		
Pgi-2	Between systems	0.002	1	0.002	0.08	0.80
	Among habitats	0.081	4	0.020	1.54	0.25
	Among locations	0.171	13	0.013	6.53	0.00
	Residual	0.021	11	0.002		
Pgm^{100}	Between systems	0.341	1	0.341	2.32	0.43
	Among habitats	0.642	4	0.160	0.83	0.53
	Among locations	2.490	13	0.192	27.43	0.00
	Residual	0.076	11	0.007		
Pgm^{93}	Between systems	0.594	1	0.594	4.87	0.19
	Among habitats	0.490	4	0.122	0.98	0.50
	Among locations	1.669	13	0.128	42.67	0.00
	Residual	0.033	11	0.003		

(N=28) drainages was 0.038. This distance is lower than the average distance of 0.047 (N=88) for comparisons involving one population from each drainage. It is also lower than the previously calculated average freshwater genetic distance, based on pairwise comparisons of all freshwater populations examined, of 0.063 (N=820). Thus, sticklebacks within watersheds do seem to be electrophoretically more similar to each other than to other freshwater sticklebacks.

The relationship of genetic distance to actual geographic distance between populations within a watershed was examined using the eleven locations sampled in the Bear River drainage. Geographic distance between sampling sites was measured along existing waterways. A regression analysis of all pairwise combinations between populations of genetic distance on geographic distance was highly significant (R^2=0.34, N=55, P<0.0001). This indicates that populations near one another in the drainage system possessed allele distributions more similar than did distant populations.

This genetic similarity between adjacent locations suggests the occurrence of gene flow, past or present, between populations. It is of interest to discover, then, if isolated populations are less genetically variable than other populations due to restricted gene exchange. To test this suggestion the eight lakes of the Bear River system (stream and swamp populations excluded) were divided into two groups. The first consisted of five lakes with 0 or 1 lake containing sticklebacks flowing into them, the second consisted of three lakes with more than 1 stickleback-containing lakes flowing into them, either

directly or indirectly (i.e. through another lake). A t test on the mean level of average heterozygosity (values arcsin square root transformed) was significant ($t=2.72$, $d.f.=7$, $P<0.05$). Isolated lakes had lower levels of average heterozygosity than lakes with greater possibilities of gene inflow. These isolated lakes in the upper regions of the watershed tend to be small lakes. In an earlier section, small lakes were found to have low levels of average heterozygosity. Thus, isolated lakes may be less heterozygous because they tend to be small, or small lakes may be less heterozygous because they tend to be isolated, or both factors may contribute to low heterozygosity.

Relationships Between Benthic And Limnetic Sticklebacks

The morphologically and ecologically distinct benthic and limnetic sticklebacks of Paxton and Enos lakes displayed strikingly divergent enzyme patterns. For both lakes, G tests indicated that allele frequencies at the Ck, Pgm and Mdh-3 loci differed significantly between benthic and limnetic populations, and in Paxton Lake significant differences in allele frequencies also existed at the Pgi-2 locus (Table 12). This indicates that in each lake the benthic and limnetic sticklebacks comprise genetically distinct populations. The genetic distance between benthic and limnetic sticklebacks in Paxton Lake was 0.1136, and in Enos Lake was 0.1263. These distances are twice as large as 0.0632, the average genetic distance between freshwater populations. Sticklebacks in Cranby, Hiechhold and Goose lakes were also polymorphic at the Ck and Pgm loci, and in Hiechhold

Table 12. Allele frequency variability between benthic and limnetic sticklebacks. G values were calculated for allele distributions at polymorphic loci. The Little Campbell comparison is betwwen the leiurus and trachurus populations. Critical chi-square values at .05 level are in brackets.

Locus		Paxton Lake[1]	Enos Lake[2]	L. Campbell River[3]
Mdh-1	Obs. G Value:	0.35	---	0.27
	Crit. Chi-square:	(3.84)	---	(3.84)
Mdh-3	Obs. G Value:	51.66***	69.18***	42.97***
	Crit. Chi-square:	(3.84)	(3.84)	(3.84)
Pgi-2	Obs. G Value:	50.27***	1.32	3.70
	Crit. Chi-square:	(3.84)	(3.84)	(3.84)
Pgm	Obs. G Value:	144.81***	172.48***	146.00***
	Crit. Chi-square:	(5.99)	(5.99)	(5.99)
Ck	Obs. G Value:	80.02***	554.30***	164.76***
	Crit. Chi-square:	(3.84)	(3.84)	(3.84)
Idh	Obs. G Value:	22.67***	21.14***	11.13**
	Crit. Chi-square:	(3.84)	(3.84)	(3.84)

** $p < 0.001$

*** $p < 0.0001$

[1] 1977 samples

[2] combined 1977 and 1978 samples

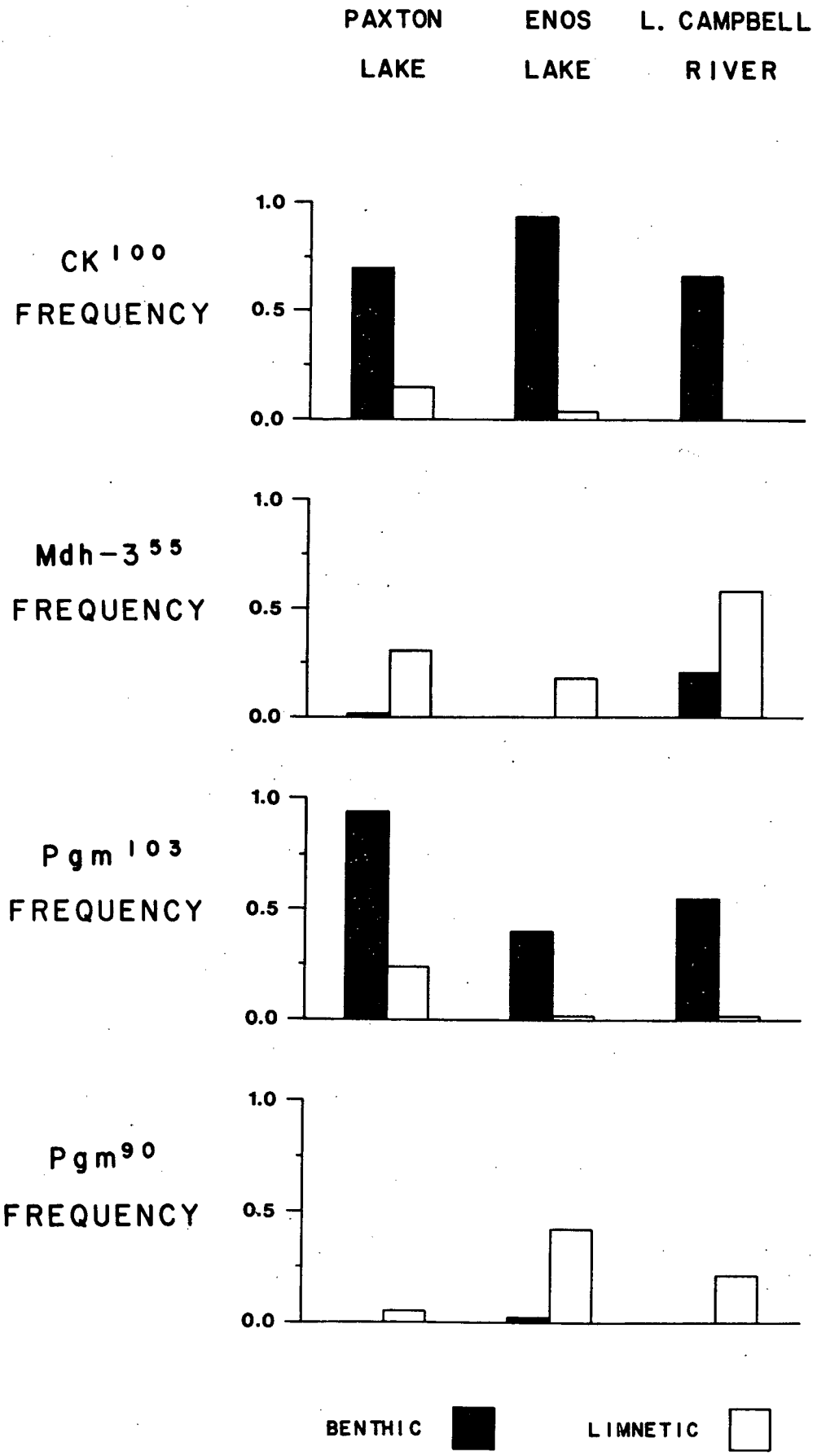
[3] 1978 samples

and Goose lakes at the Mdh-3 and Pgi-2 loci as well. Allele frequency differences between phenotypes were not examined in these lakes.

Morphologically distinct populations of sticklebacks also come in contact in many streams in which both trachurus and leiurus populations breed in the spring and early summer. In the Little Campbell River, the breeding regions overlap and some interbreeding apparently occurs (Hagen 1967). Morphologically, the trachurus bear a closer resemblance to the limnetic than to the benthic sticklebacks of Paxton and Enos lakes. They are terete and heavily armoured, and possess the long and numerous gill rakers and silvery counter-colouration characteristic of fish leading a pelagic existence. The Little Campbell leiurus, on the other hand, resemble the lacustrine benthic sticklebacks, both in their deep body shape and reduced armour as well as in dark colouration. The amount and nature of the genetic differentiation between the leiurus and trachurus sticklebacks populations was very similar to that between the lacustrine benthics and limnetics. Allele frequencies at the Ck, Pgm and Mdh-3 loci differed significantly between the two types (Table 12), and Pgi-2 was slightly polymorphic in the marine but not the freshwater population. The genetic distance between the leiurus and trachurus populations was 0.1006, greater than the average distance of 0.0608 between marine and freshwater populations.

Figure 6 illustrates that in each of the pairs of populations, not only were the same loci polymorphic and different between the two morphs, but the differences in allele

Figure 6. Allele frequency differentiation between benthic and limnetic sticklebacks. The Little Campbell comparison involves the trachurus (limnetic) and leiurus (benthic) populations.


PAXTON LAKE
ENOS LAKE
L. CAMPBELL RIVER
CK 100 FREQUENCY
Mdh-3 55 FREQUENCY
Pgm 103 FREQUENCY
Pgm 90 FREQUENCY
1.0
0.5
0.0
BENTHIC
LIMNETIC

frequencies were in the same direction. Thus, the frequency of the Ck^{100} allele was higher in all three benthic populations, the frequency of $Mdh\text{-}3^{55}$ was higher in all three limnetic populations, the Pgm^{103} allele was more common in benthic and Pgm^{90} in limnetic populations. While the average genetic distance between each of the benthic-limnetic pairs was 0.1135, the average distance between the three benthic populations was 0.0275 and between the three limnetic populations was 0.0263.

Discussion

The electrophoretic survey of G. aculeatus populations indicates the morphological variability characteristic of this species is accompanied by considerable heterogeneity at the molecular level. Six of eight enzyme loci examined exhibited genetically controlled variability in isozyme banding patterns. Although eight is a small number of loci on which to base estimates of polymorphism and heterozygosity for use in interspecific comparisons, the primary purpose of this study was to examine intraspecific relationships.

With the exception of creatine kinase, which had been studied previously in Gasterosteus (Hagen 1967, Michiel 1977), none of the loci examined were known to be polymorphic when the study began. The remaining seven enzymes were chosen by virtue of clear resolution of the isozyme bands, and the subsequent confirmation of their genetic control. Thus, the estimates of genetic variability derived from gene frequencies at these loci are unbiased by conscious selection for heterogeneity. Nevertheless, at least three of the enzymes used in the present

study are notably polymorphic in fish species: CK (Ferris and Whitt 1978), PGI (Avise and Kitto 1973) and PGM. Therefore, a disproportionate number of polymorphic loci may be included. In a recent study, Avise (1976) examined variability at as many as 15 loci in three California Gasterosteus populations. In addition to polymorphism at loci examined in this study (Mdh-1, Mdh-3 (his Mdh-2), Pgi-2, Pgm and Ck (his Pt-3?)), he reported variability at an esterase locus, Est-1, a triosephosphate isomerase locus, Tpi-1, and a general protein locus, Pt-1.

The inclusion of a large proportion of atypically variable loci accounts, at least in part, for the high average values of heterozygosity and polymorphism indicated by the data. The values of 0.124 for heterozygosity and 0.466 for polymorphism are considerably greater than the mean values of 0.051 and 0.152 respectively, calculated for 51 species of Osteichthyes (Nevo 1978). In that review, the only species with values exceeding those of the present study was the killifish, Fundulus heteroclitus (Mitton and Koehn 1975). Considerably lower estimates of both heterozygosity (0.09) and polymorphism (0.27) for Gasterosteus were obtained by Avise (1976), and although based on samples from a single population, were derived from information at 15 genetic loci.

No deviations from Hardy-Weinberg conditions were found in the genotypic distributions at any polymorphic locus beyond those attributable to sampling error. Close agreement between observed and expected genotypic proportions is the rule rather than the exception in analyses of electrophoretic gene frequencies, and more likely reflects the weakness of the test

(Workman 1969, Ward and Sing 1970) rather than precludes the possibility of selection or non-random breeding within the examined populations. In a detailed study of an eelpout (Zoarces viviparus) population, Christiansen et al. (1973) found that post-zygotic selection favoured Est-III homozygotes at the expense of individuals heterozygous at that locus. Although no significant deviation from Hardy-Weinberg conditions was apparent, there was a significant deficiency of Est-III heterozygotes revealed among the adult eelpout population by more powerful methods of mother-offspring analysis, and later by age group analysis (Christiansen et al. 1974).

The observed stability of gene frequencies over short time periods in this study is also characteristic of other studies on fish populations (Allendorf and Utter 1979, Avise and Felley 1979) to the limited extent that temporal variability has been examined. However, studies with other organisms (Gaines et al. 1978, Berger 1971, Dobzhansky and Ayala 1973) have indicated that temporal genetic variability, mediated by selection, does occur within populations. The change in the frequency of the Mdh-3^{100} allele in the Serpentine River (TYNEP) from 0.26 in March 1978 to 0.06 in June 1979 may reflect such a process, but confirmation would require investigation of the population structure and the identification of the selective agent(s).

Small differences in allele frequencies at the Mdh-3 and Ck loci between 1977 and 1978 samples of Enos Lake limnetics may reflect real changes in the genetic composition of the population, but more likely resulted from the inclusion of benthic or hybrid fish in the 1977 sample. In that year, fish

were not separated on a morphological basis before electrophoresis, but merely by method of capture (minnow trap or night-time seine haul) and some misclassification likely resulted.

Failure to recognize gene frequency changes in other populations may reflect the short time intervals between sample collections, or sample sizes too small to reveal the significance of slight, but real, changes. Lack of standardization of the age and sex composition of repeated samples may also obscure real shifts in gene frequencies that occur over time.

While the disproportionate number of polymorphic loci included in my study hinders comparisons of genetic variability with other species, it facilitates examination of relationships among conspecific populations. Both monomorphic loci, Ldh and Idh, are fixed for the same allele in all populations, marine and freshwater, and provide no information on the genetic relationship of any population to another. The degree of differentiation at polymorphic loci, on the other hand, can be used as a measure of genetic distance between populations, and from such data evolutionary inferences are often drawn.

Nei's genetic distance, D, constitutes a measure of the accumulated number of gene substitutions per locus between two populations, which, if the rate of gene substitution per year is constant (i.e. time-dependent), is linearly related to evolutionary time (Nei 1972, 1975). Increasing evidence for the selective value of some electrophoretic variability demonstrates convincingly that heterogeneity at the enzyme level is not all

selectively neutral, nor controlled entirely by population parameters and stochastic events over time. Significant correlations between enzyme genotypes and environmental variables (such as temperature) are known for a number of fish species (Sick 1965, Koehn and Rasmussen 1967, Johnson 1971, Frydenberg et al. 1973, Nyman 1975, Mitton and Koehn 1975) as well as for many other organisms ranging from snails (Johnson 1976) to ants (Tomaszewski et al. 1973) to barley (Hamrick and Allard 1975). In some cases, functional characteristics of the different alleles at a locus have been examined, and the optimal performance conditions of each allelic product found to correspond to the natural environment in which the allele commonly occurs (Merritt 1972, Koehn 1969). To the extent that electrophoretic variation is subject to natural selection, measures of genetic distance provide good estimates of the genetic differentiation among populations, but not of their evolutionary relationships.

In the present study, relationships among and between marine and freshwater sticklebacks are examined on the basis of genetic distance. Bell (1976) postulated that the freshwater populations in regions including the present study area are the result of postglacial polyphyletic evolution from the marine trachurus form. He thus suggested that electrophoretic genetic heterogeneity should be greater within trachurus than leiurus populations, owing to their greater age and lesser vulnerability to the erosion of genetic variability through founder effects and genetic drift. On the other hand, genetic heterogeneity between the interconnected marine populations should be less

than between those occupying disjunct freshwater localities. Thus, while marine populations should be genetically similar by virtue of gene flow, the independently established freshwater populations should bear no greater resemblance to each other than to the marine populations from which they descended.

Although there is no way of establishing the degree of similarity between gene frequencies in extant marine populations and those characterizing postglacial marine populations, the present nature and degree of electrophoretic variability in marine sticklebacks is compatible with the idea that all freshwater populations have a marine origin. The marine populations possess (usually at low frequencies) all the electrophoretic variants detected in freshwater populations. Even the band defined by Hagen (1967) as "diagnostic" for the marine form in the Little Campbell River has been found in lake populations (unpub. data); however, it does seem to be absent from resident stream-dwelling populations such as the one Hagen examined.

In addition, the results of the present study confirm a number of Bell's (1976) predictions. The average genetic distance between marine populations is 0.0055 while between freshwater populations it is 0.0632. Thus, there is over ten times more distance between freshwater than between marine populations. As predicted, the smaller distances between marine populations indicate that they are genetically more homogeneous than freshwater populations. Moreover, the average distance between marine and freshwater populations is slightly less than the average between pairs of freshwater populations (0.0608).

Again, as predicted, freshwater populations are no more similar to one another than they are to marine populations.

The average number of polymorphic loci in marine populations is 4.8 (60%), and in freshwater populations it is 3.4 (43%). This supports the contention that intrapopulation heterogeneity should be greater within marine than within freshwater populations. In contrast, however, average heterozygosity is slightly higher in freshwater (0.126) than in marine (0.118) populations. Thus, while the marine environment possesses the entire range of electrophoretic variants, these variants occur at uniformly low frequencies in marine populations.

These results concerning the intra- and interpopulation variability in marine and freshwater populations can also be explained in another way. If the tenuous assumption of selective neutrality made by Bell (1976) is discarded, measures of genetic distance are no longer necessarily related to evolutionary time in a linear fashion. In Gasterosteus, the greater electrophoretic heterogeneity among freshwater populations may result from the large variety of selective regimes imposed by diverse freshwater environments and the absence of gene flow among these disjunct populations. The electrophoretic homogeneity among marine stickleback populations may result from the relative uniformity of marine environments combined with the possibility of gene flow among them. Thus, the large genetic distances among freshwater populations, and between freshwater and marine populations, may reflect the large differences in selective forces among these environments rather than the long

evolutionary isolation of populations occupying them.

A comparison of allelic differentiation between the Somass (west coast of Vancouver Island) and Bear (east coast) river systems reveals that neither drainage system nor habitat type accounts for a large amount of the great among population variability in allele frequencies found in the 19 locations sampled. Only the frequency of Mdh-1^{100} is significantly heterogeneous among habitats. This is due to relatively high frequencies of the variant allele Mdh-1^{82} in large lakes as compared to small lakes.

Nevertheless, genetic distances are slightly less between populations from the same drainage (Bear or Somass) (0.038), than between populations from different drainages (0.047). This provides some evidence for intradrainage homogeneity; however, the degree of genetic distinctness of Gasterosteus within the two watersheds was much less than found by Hedgecock (1978) in a similar comparison between watersheds of salamander (Taricha rivularis) populations. For Taricha, the average between drainage genetic distance, although less than both the between and within drainage distances for sticklebacks, was almost four times as great as the average within drainage distance.

In contrast, results similar to mine were reported by Avise and Smith (1974) and Avise and Felley (1979) for southeastern U. S. reservoir populations of bluegill (Lepomis macrochirus). Within macrogeographic areas, these investigators found relative homogeneity in allele frequencies at polymorphic loci within populations (reservoirs), and great genetic heterogeneity among reservoirs within a drainage system. There was no significant

increase in allele frequency variance in comparisons between drainage systems (Avise and Smith 1974). There was, however, genetic differentiation between drainages in three different macrogeographic regions inhabited by two separate subspecies of L. macrochirus and their hybrids.

While the lack of genetic distinction between the Somass and Bear river systems fails to provide convincing support for the suggestion of independent evolution within drainages, data from a larger number of more geographically widespread river systems are required to rigorously test the hypothesis. It might be argued that the close proximity of the two watersheds in this study indicates a common postglacial history, with a high probability of simultaneous colonization by closely related marine populations. The fact that even the between drainage genetic distance of 0.047 is less than the average distance of 0.063 between all freshwater populations supports this suggestion. However, Mathews et al. (1970) suggest that marine invasion in the Alberni inlet on the west coast (into which the Somass River flows) may have occurred significantly before invasion of the eastern side of Vancouver Island (on which the Bear system lies). The retreat of glacial ice in an easterly direction is responsible for the difference in timing.

Even if the amount of genetic variation between watersheds is generally greater than that distinguishing the Bear and Somass systems, there obviously exists another source of variability underlying the large amounts of genic heterogeneity observed between localities within a single drainage. It is possible that electrophoretic differentiation within, as well as

between, watersheds is a reflection of founder effects and genetic drift.

Geological evidence indicates that with the retreat of the last major glaciation from Vancouver Island and the lower Fraser Valley (approximately 13,000 years ago) land levels rose rapidly. Mathews et al. (1970) state: "... the first 300 ft of emergence (out of an ultimate uplift of perhaps 700 ft) occurred within a few hundred years, and the first 500 ft in not more than 1,000 years". At present, natural populations of sticklebacks on Vancouver Island and in the Fraser Valley occur only at elevations up to 700 ft (J. D. McPhail, pers. comm.), and maximum elevations decrease on southern Vancouver Island where Mathews et al. (1970) indicate postglacial land emergence was least. A subsequent, less severe submergence of land, and accompanying marine transgression of terrestrial habitats, occurred during a minor glacial advance approximately 11,000 years ago. This provided an opportunity for secondary marine invasion at lower elevations. The following re-emergence of land to levels not greatly different from those of today was complete about 9,000 years ago. Levels have varied little (about 35 ft) since that time (Mathews et al. 1970).

Small numbers of the founding marine populations, or their early descendants, were likely isolated in suitable habitats within drainages as postglacial land levels rose and sea levels dropped. The significant correlation between geographic and genetic distance within the Bear system may be the result of restricted gene flow between adjacent localities as water levels subsided. Certainly the small lakes at higher elevations were

the first to be isolated, and the low levels of heterozygosity in the headwater lakes of the Bear drainage are consistent with the concept that gene flow at that time affected the present genetic structure of populations.

Larger founding populations, greater gene flow among them and, possibly, secondary invasion by marine populations, may all have contributed to the present high levels of heterozygosity in the large lakes and low-lying streams occupying the lower reaches of watersheds. The present striking differentiation among populations within drainages may be the result of genetic drift over the past 9,000 years (while sea levels have been stable) compounding the original founder effects among localities. Certainly, extreme morphological differentiation over short distances attests strongly to the effectiveness of regions of poor habitat as barriers to present day gene flow.

Thus the observed patterns in heterozygosity levels can be attributed to historical events affecting gene flow within and between populations. Merritt et al. (1978) attributed a similar cline in level of heterozygosity in longnose dace (Rhinichthys cataractae) populations of the South Connecticut River to the stochastic processes of founder effect and drift. Avise and Felley (1979) also emphasized the importance of breeding structure and gene flow in their study of intradrainage electrophoretic variability in the bluegill. However, they noted: "... it may not be unreasonable to propose that selection differentials between reservoirs are far greater than those within, particularly since roughly parallel clines of allele frequencies occur across these two presently isolated

drainages".

Convincing arguments can also be made for the role of selection in bringing about the patterns of genetic variability displayed by Gasterosteus populations. Low levels of heterozygosity are found to characterize not only the small lake populations of the Bear and Somass systems, but the populations of small lakes and isolated streams in general.

The two most likely explanations for this lack of genetic variability are (1) founder effects, and genetic drift in small post-founding populations or during population bottlenecks, as discussed above, or (2) directional selection imposed by homogeneous environmental conditions (i.e. lack of niche variablity).

Founder effects and genetic drift have likely affected the tiny populations of isolated streams and swamps. These occupy habitats less than 0.01 km^2 in area, and are continually subjected to environmental stress (such as winter freezing and summer drought). Population levels likely range between 10^2 and 5×10^3 during spring and summer breeding, but overwinter survival is minimal.

Small lakes, on the other hand, are generally much larger (up to 1 km^2 in this study) and provide considerable buffering from climatic extremes. Population levels reach 10^4 to 10^5 or more during summer months, and, although winter mortality may be high, especially among adults, population levels probably do not drop to those at which genetic drift becomes important. Mark and recapture estimates of population size of the introduced Marion Lake sticklebacks indicated that the 4,000 sticklebacks planted

in the summer of 1974 had increased to 60,000 in the summer of 1975, to over 100,000 in the summer of 1976 and have averaged around 60,000 in subsequent years. It appears unlikely, then, that genetic drift constitutes the mechanism responsible for reduced electrophoretic variability in small-lake Gasterosteus populations.

Although founder effects cannot be dismissed as a possible factor, the large amounts of morphological differentiation that occurred in the process of deriving present day freshwater populations from the original marine form indicate that levels of genetic variability in founding populations were not extremely low. Gorman et al. (1975) working on island populations of adriatic lizards calculated that a single pregnant female would introduce 34% of the species variability into a new population. This illustrates the very small sizes of founding populations required for founder effects to be pronounced.

The structural similarity in habitats occupied by island populations of terrestrial organisms and lake populations of freshwater organisms was noted by Avise and Smith (1974). These authors suggested that the genetic distinction of lake populations, like that of island populations, is the result of increased genetic drift under conditions of geographic isolation imposed by the physical barriers of land, in the case of lakes, and water, in the case of islands. Sticklebacks, occupying both the ocean and isolated freshwater habitats, are distributed remarkably like those terrestrial organisms that populate both mainland and island sites.

Gorman et al. (1975) found that patterns in heterozygosity levels similar to those of the present study characterized mainland and island populations of the lizard Lacerta sicula. These lizards possess a number of features in common with Gasterosteus. They exhibit broad ecological tolerance, and are both polytypic (displaying striking variability in colour and morphology between populations) and polymorphic (possessing within population variability for a number of morphological traits).

Island (the terrestrial equivalent of small lake) populations possessed lower levels of heterozygosity than did mainland (terrestrially equivalent to marine) populations. The lizards on very small islands (< 0.01 km^2), like the sticklebacks of isolated streams and swamps, were even less heterozygous. As in the present study, this "small island effect" in the smallest of populations was attributed, at least in part, to genetic drift and founder effects. Gorman et al. (1975) noted, however, that "although genetic drift might account for the possible loss of alleles in the smallest fringing populations, this in no way implies that the alleles are behaving neutrally".

On other islands (> 0.05 km^2) Gorman et al. attributed the higher, but still low levels of heterozygosity to selection. They proposed the "time-divergence" hypothesis to explain their results (Soule and Yang 1973, Gorman et al. 1975). Specifically, they felt that "(1) genetic variability is lost as a consequence of directional selection at rates proportional to average evolutionary rates and (2) evolutionary rates of island

reptiles everywhere seem to be inversely proportional to island size because (3) the relative ecological distinctness is greater on small islands than large ones".

In many respects, the small lakes inhabited by Gasterosteus constitute an environment that is apparently less spatially and trophically homogeneous than the islands occupied by the Lacerta lizards. The fact that Paxton and Enos lakes each contain two ecologically and morphologically distinct stickleback populations supports the observation of habitat heterogeneity. This phenomenum of co-existing benthic and limnetic populations is associated with low numbers of predators in both lakes (J. D. McPhail, pers. comm.). Typically, small lakes inhabited by Gasterosteus in the study region also are inhabited by stickleback predators and competitors. These include: trout (Salmo clarki and S. gairdneri), sculpins (Cottus spp.), and often salmon (Oncorhynchus spp.), squawfish (Ptychocheilus oregonensis) and other species. In many small lakes, predation likely restricts the type of habitat Gasterosteus can exploit.

Under such conditions, sticklebacks tend to lead a cryptic, benthically-oriented existence. The limnetic form found in Paxton and Enos lakes occupies, and feeds in, the pelagic zone. It builds nests in exposed areas and provides an obvious target for predation. Indeed, the introduction of coho salmon (O. kisutch) into Paxton Lake led to a drastic reduction in numbers of limnetic sticklebacks (Larsen 1976, McPhail, pers. comm.). Thus, the actual habitat available for Gasterosteus in most small lakes may be more narrowly defined than the physical and trophic properties of the lakes suggest.

The loss of genetic variability through directional selection, much of it undoubtedly applied through predation, is a distinct possibility in these lakes.

The Gasterosteus populations of large lakes do not exhibit the same reduction in electrophoretic variability relative to marine populations. While the degree of polymorphism in large-lake sticklebacks is slightly lower than in marine populations, the level of heterozygosity is slightly higher. These results correspond closely with those of Gorman et al. (1975) who found that populations of Lacerta melisellensis on large islands displayed high levels of heterozygosity. They attributed maintenance of this variability to the greater ecological variety (i.e. greater niche width) on large islands.

Certainly, the large lakes of this study provide a much greater range of habitat diversity than do the small lakes; the size of pelagic regions, especially, increases disproportionately with lake surface area. However, large lakes possess the same range of predators and competitors as small lakes, and to assume greater stickleback exploitation of the pelagic zone in large lakes one must postulate reduced interspecific interaction. Manzer (1976) in a dietary study of Gasterosteus and juvenile sockeye salmon (O. nerka) in Great Central Lake found considerable overlap in food consumed by these two species, but concluded that serious competition did not exist. Competition and predation undoubtedly occur in large lakes, but the extent depends to a large degree on the amount of spatial and temporal segregation among species. The opportunities for such segregation probably are greater in large

than in small lakes.

Thus, it seems likely that the absence of reduced electrophoretic variability in large lakes is due, at least in part, to the range of habitats available. The morphological similarity between the sticklebacks of large lakes and marine populations (Hagen and Gilbertson 1972) may indicate similarity in the selective regimes in the two types of habitat. Although, according to the time-divergence theory, this morphological similarity may not result so much from similar selective regimes as from the absence of directional selective forces. The consequent lack of differentiation, or slow evolutionary rate, results in the maintenance of genetic variability.

Since marine populations are highly polymorphic, the observation that heterozygosity is greater in large lakes than in the ocean indicates that the variant alleles occur at higher frequencies in large lakes. Whether this reflects a reduction in the stringent selection against these alleles that keeps them at uniformly low frequencies in marine populations, or results from actual selection for the variants in large lakes is not clear.

Perhaps more surprising than the high levels of heterozygosity in large lakes are the even higher levels characterizing populations inhabiting low-lying streams and swamps. These are much smaller populations, occupying such habitats as tributaries to the Fraser River, or small streams and ponds draining directly to the ocean as in Sooke, or swamps adjacent to and often connecting the lakes of large watersheds. Temporal, rather than spatial, heterogeneity of the environment is most likely responsible for maintaining genetic variability

in these populations. Many of the coastal populations occupy habitats under tidal influence. Others, more distant from the ocean, are subject to the flooding typical of lowland regions. All are vulnerable to climatic fluctuation, somewhat ameliorated by lower elevation, as are the more ephemeral isolated stream and swamp populations. Despite both random and predictable habitat variability over time, the generally larger population sizes in these low-lying streams (compared with those of isolated stream localities) undoubtedly reduce the erosion of genetic heterogeneity through genetic drift.

Another factor that may help maintain variability is gene flow. The Fraser River constitutes a permanent dispersal route for populations occupying its tributaries, and flooding likely creates transient connections among other low-lying populations. However, if such gene flow does occur, it is insufficient to swamp the genetic differentiation that is apparent, even between many geographically adjacent sites. This may reflect the strength of the selective forces affecting gene frequencies in these populations. Such forces, unlikely to be directional, probably vary over time with environmental fluctuation.

The present analysis reveals further evidence for the influence of natural selection on gene frequencies. If freshwater populations are of marine origin, and the founding genomes were truly representative of marine gene frequencies, then the average gene frequencies of present day marine and freshwater populations should be the same, unless they are altered by selection. The data examined to this point are compatible with the suggestion of evolution of freshwater

populations from marine sticklebacks through isolation in freshwater habitats. If this is the case, loci monomorphic in marine populations should be monomorphic for the same allele in freshwater populations. Loci polymorphic in marine populations may be polymorphic or monomorphic (through founder effects) in freshwater ones. If some freshwater populations are monomorphic at a locus, then in freshwater habitats the frequencies of fixation for alleles at that locus should be proportional to their frequencies in marine populations.

As predicted, the two monomorphic loci, Ldh and Idh, are fixed for the same allele in all populations (marine and freshwater); however, allele frequencies at two polymorphic loci, Pgm and Mdh-3, differ significantly between the two environments. At the Pgm locus, marine populations are characterized by high frequencies of the most common allele, Pgm^{100}, lower frequencies of Pgm^{90} and relatively rare occurrences of other alleles. While Pgm^{100} is also the most common allele in freshwater populations, other alleles, Pgm^{80}, Pgm^{90} and Pgm^{103}, are often present at as high, or higher, frequencies. Pgm is fixed (monomorphic) in only two samples (Appendix VII), both from freshwater, and in both cases the locus is fixed for the common Pgm^{100} allele. No significant differences in allele frequencies have been demonstrated among freshwater populations occupying different habitats.

At the Mdh-3 locus, $Mdh\text{-}3^{55}$ predominates in marine and $Mdh\text{-}3^{100}$ in freshwater populations. Thus, average gene frequencies differ between marine and freshwater habitats. Moreover, while the average frequency of $Mdh\text{-}3^{55}$ is 0.853 and of $Mdh\text{-}3^{100}$ is

0.147 in marine populations, only three freshwater populations are fixed for Mdh-3^{55} and seven (excluding the introduced Marion Lake population) are fixed for Mdh-3^{100} (Appendix II). Although selection is implicated as the force underlying the marine-freshwater allele frequency dichotomy, no significant heterogeneity in Mdh-3 allele frequencies is apparent among freshwater populations occupying different habitats. This indicates that the selection resulting in marine-freshwater Mdh-3 differentiation is associated with some fundamental difference between marine and freshwater existence. Presumably, this difference is imposed either externally by the environment or internally by physiological constraints.

Raunich et al. (1972) reported a similar distribution for a genetically controlled hemoglobin variant in European populations of Gasterosteus. They examined resident freshwater populations in Italy and Germany and brackish water populations in the coastal estuaries and lagoons of the northwestern Adriatic Sea. While freshwater populations were monomorphic for the common hemoglobin A, coastal populations exhibited high frequencies of hemoglobin B.

Although overall allele frequencies at the Ck locus did not differ between marine and freshwater populations, the frequency of Ck^{100} was significantly higher in populations inhabiting low-lying streams and swamps than any other habitat, including the ocean. Ck^{100} was universally present in freshwater populations occupying streams that receive spring breeding populations of marine sticklebacks in their lower reaches. Thus it was present not only in the resident sticklebacks of the Little Campbell (LC

18), Sarita (SARIJ) and Chehalis (Washington) (CHEHW) rivers, but also in the low-plated (leiurus) fish of mixed leiurus-trachurus samples collected from the Fraser River (FRASR), and from Lard (LARDC) and Fuller (FULCR) creeks on Vancouver Island. Freshwater populations in tributaries to the Fraser (GIFFS and TEXAS) also possessed the allele. It was also present in resident populations of streams and rivers from which marine sticklebacks were not sampled but likely enter, such as the Colquitz (CLQTZ), Serpentine (TYNEP) and Tugwell (SKOOS) sites in B.C., and the North Skagit (SKAGI) and Snohomish (EBEYI) sites in Washington. Although the low-plated populations were in some cases many kilometers upstream from regions occupied by marine spawners, the presence of Ck^{100} seems definitely associated with use of a stream by the two types of stickleback populations.

In contrast, this allele is almost completely absent from lake populations regardless of lake size (exceptions are Sarita and Trout lakes). Small lakes that possess two completely or partially distinct stickleback populations (Enos, Paxton, Goose, Cranby and Hiechhold) also are exceptions. In Enos and Paxton, Ck^{100} is again present in high frequencies in the deep-bodied benthic sticklebacks and almost absent among the limnetics. However, the benthic-like sticklebacks of other small lakes, in the absence of of the limnetic form, do not generally possess the Ck^{100} allele (Appendix VI). Thus, for Ck allele frequencies, as for certain morphological traits (Hagen and Gilbertson 1972), populations of low-plated sticklebacks in lakes distant from the ocean are more similar to marine sticklebacks than are low-

plated populations inhabiting streams in which marine sticklebacks also breed. Although the mechanism underlying this apparent case of character displacement at the enzyme level is unknown, the phenomenum appears widespread and may be characteristic of trachurus-leiurus interactions.

The highly significant correlations of Ck^{85}, Pgm^{103} and $Pgi\text{-}2^{100}$ frequencies in marine populations with longitude result from small, but consistent, differences in allele frequencies between marine populations from the west coast of Vancouver Island and those of the more easterly Strait of Georgia. The frequency of the common Ck^{85} and $Pgi\text{-}2^{100}$ alleles are lower, and of the rare Pgm^{103} is higher, in the west coast populations (Appendices V, VI and VII). This reflects the slightly greater genetic heterogeneity characterizing these populations.

There are several possible explanations for this genetic differentiation: (1) selective differences between the regions, (2) founder effects (i.e. differential postglacial invasion from northern and southern refugia) or (3) genetic drift, if gene flow between the regions is low. Understanding of the actual mechanisms might be aided by the determination of allele frequencies in samples from more northern and southern extremes of the trachurus range.

The striking electrophoretic differentiation between benthic and limnetic populations within Paxton and Enos lakes, coupled with the electrophoretic similarity between the two benthic and two limnetic populations, corresponds with morphological patterns of varibility. If evolution of the two forms occurred independently within each of the lakes the

evidence for the selective value of the electrophoretic variants under such conditions is impressive, especially as preliminary evidence reveals no correlation between inheritance of the morphological and electrophoretic variability. (However, the possibility of the linkage of morphological and electrophoretic loci is deserving of closer examination.)

Genetically distinct 'species pairs' of fish, morphologically and electrophoretically differentiated, occur in a number of lakes. Kirkpatrick and Selander (1979) studied morphologically distinct sympatric populations of whitefish (Coregonus clupeaformis) in the Allegash Basin, Maine. Lakes of the region were populated by one or both of two whitefish morphs, a normal and a dwarf. These forms differed not only in growth rate but also in a number of meristic traits. Allele frequencies at several electrophoretic loci indicated that the two forms within a lake were genetically isolated. Normal, or dwarf, populations from different lakes were not, however, electrophoretically more similar than normal and dwarf populations from the same or different lakes. Kirkpatrick and Selander (1979) interpreted the presence of similar C. clupeaformis dwarf-normal species pairs in at least two other widely separated regions of North America (Squanga Lake, Yukon Territory and Lake Opeongo, Ontario) as evidence of several independent evolutions of the two "species".

Similar studies on brown trout (Allendorf et al. 1976) and Arctic char (Henricson and Nyman 1976, Nyman 1972, Nilsson and Filipsson 1971) in Scandinavian lakes revealed the presence of genetically discrete species pairs. Henricson and Nyman (1976)

compared gene frequencies in allopatric and sympatric populations of two types of Arctic char which occurred by themselves in some lakes and co-existed (as a species pair) in other lakes. They found that, in sympatry, low rates of gene flow between the two types had led to introgression at electrophoretic loci, but as yet had not obscured their genetic distinctness. It is not clear, for any of the species examined, whether the two co-existing forms evolved in sympatry or during geographical isolation.

The possibility of the double invasion of both Paxton and Enos lakes by common populations of benthic and limnetic sticklebacks during postglacial water level fluctuations cannot be disproven. In that case, the electrophoretic affinities of the two benthic and two limnetic populations might simply be a reflection of common ancestry rather than a response to similar selective regimes. However, more support for the selective theory is provided by the genetic similarity between these lacustrine benthic and limnetic sticklebacks and their lotic counterparts, the Little Campbell leiurus and trachurus populations. Again, ecological and morphological similarities correspond closely to electrophoretic similarity, and in this case the possibility of common origin of similar phenotypes is more remote.

Whatever their origin, allele frequencies of the adult benthic and limnetic sticklebacks from Enos and Paxton indicate that within each lake the two morphs constitute genetically isolated populations. As in the sympatric species of Scandinavian char, extremely low rates of gene exchange may take

place. Hybridization between the two morphs occurs in Paxton Lake (J. D. McPhail, pers. comm.) but few genomes among the adults surveyed were of possible hybrid origin, indicating that hybrids face a severe selection differential in the natural environment. Lab-reared hybrids display the expected highly heterozygous genotypes. Thus, like the leiurus and trachurus sticklebacks of the Little Campbell and other streams (Hagen 1967), benthic and limnetic sticklebacks act as good biological species.

These results contrast sharply with those of Avise (1976) who examined allele frequencies at electrophoretic loci in a Gasterosteus population dimorphic for plate counts (high and low) in the San Joaquin River, California. Unlike the lacustrine benthics and limnetics of the present study, these two types did not differ in other morphological characteristics such as body size and shape. Nor were electrophoretic allele frequencies at polymorphic loci different between the high and low plated forms, and inheritance of plate phenotypes in lab crosses supported the suggestion that the two types constituted a single interbreeding population.

The final data pertaining to the influence of selection on gene frequencies at the enzyme level in Gasterosteus is provided by a comparison of allele frequencies in Chemainus and Marion lakes. Gene frequencies were not determined in Chemainus Lake prior to, nor in either lake immediately following, the 1974 introduction of Chemainus sticklebacks into Marion. However, allele frequencies at all loci, including the polymorphic Pgm, Mdh-1 and Pgi-2, are not significantly heterogeneous among the

four 1977 and 1978 Chemainus and 1977 and 1979 Marion samples. Any changes that have taken place in Chemainus Lake at electrophoretic loci since 1974 have occurred independently in Marion Lake. On the other hand, morphological variability has been monitored in both lakes since 1974. Gradual, but significant, change in the degree of asymmetry of, and mean value of, plate counts has occurred in Marion Lake, but not Chemainus (J.D. McPhail, pers. comm.). This indicates that if electrophoretic variability at the loci examined in this study is subject to selection, it responds to different, or more slowly to the same, environmental variables than do morphological traits.

Summary

Levels of heterozygosity and polymorphism in 79 populations of G. aculeatus are comparable to those characteristic of other vertebrate species. In general, genotypic distributions conform with Hardy-Weinberg expectations. While gene frequencies at polymorphic loci are stable over time within populations, they are highly heterogeneous among populations.

Genetic distances are much lower among marine than among freshwater stickleback populations. The distances between marine and freshwater populations are similar to those among freshwater localities. These findings are compatible with the suggestion that freshwater (leiurus) populations in southwestern B. C. are polyphyletic, and have descended from marine (trachurus) sticklebacks isolated in freshwater habitats during postglacial fluctuations in water levels. Alternatively, these results can

be explained by postulating differences in selective forces among freshwater, and between marine and freshwater, environments. Comparisons of genetic variability within and between the Bear and Somass river watersheds do not provide strong support for either hypothesis.

Although trachurus populations are genetically more homogeneous than leiurus populations, a clear geographic distinction in allele frequencies at the Pgm, Pgi-2 and Ck loci separates marine sticklebacks inhabiting waters off the west coast of Vancouver Island from those occupying the Strait of Georgia. No consistent geographic patterns in allele frequencies are apparent among the highly heterogeneous freshwater populations.

Gasterosteus populations inhabiting the ocean, large lakes and low-lying streams are more polymorphic and heterozygous than those occupying small lakes and isolated streams. Both stochastic (founder effects and genetic drift) and deterministic (natural selection) factors can be invoked to account for these patterns. Their relative contributions to present levels of variability remain speculative.

Evidence for the effect of selection on allele frequencies includes differences in allele distributions at the Pgm and Mdh-3 loci between marine and freshwater populations, and elevated frequencies of Ck^{100} in the leiurus populations of low-lying streams. The similarity of electrophoretic and morphological affinities among the benthic and limnetic 'species pairs' in Paxton and Enos lakes, and the leiurus and trachurus sticklebacks of the Little Campbell River, is also suggestive of

a selective influence on electrophoretic variation. However, morphological divergence between the sticklebacks of Chemainus and Marion lakes is as yet unaccompanied by allele frequency differentiation, indicating that electrophoretic variability may be less responsive to environmental change than is morphology.

LITERATURE CITED

Allendorf, F. W., N. Ryman, A. Stennek and G. Stahl. 1976. Genetic variation in Scandinavian populations of brown trout (Salmo trutta L.): evidence of genetically distinct sympatric populations. Hereditas 82: 19-24.

Allendorf, F. W., F. M. Utter and B. P. May. 1975. Gene duplication within the family Salmonidae. II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations. In: Isoenzymes IV. Genetics and Evolution (C. L. Markert, ed.), pp. 415-432.

Allendorf, F. W. and F. M. Utter. 1979. Population genetics. In: Fish Physiology Vol. VII. Bioenergetics and Growth (W. S. Hoar, D. J. Randall and J. R. Brett, eds.), pp. 407-454. Academic Press.

Avise, J. C. 1976. Genetics of plate morphology in an unusual population of threespine stickleback (Gasterosteus aculeatus). Genet. Res., Cambridge 27: 33-46.

Avise, J. C. and J. Felley. 1979. Population structure of freshwater fishes I. Genetic variation of bluegill (Lepomis macrochirus) populations in man-made reservoirs. Evolution 33: 723-739.

Avise, J. C. and G. B. Kitto. 1973. Phosphoglucose isomerase gene duplication in the bony fishes: an evolutionary history. Biochem. Genet. 8: 113-132.

Avise, J. C. and M. H. Smith. 1974. Biochemical genetics of sunfish I. Geographic variation and subspecific

intergradation in the bluegill, Lepomis macrochirus. Evolution 28: 42-56.

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourao and S. Perez-Salas. 1972. Enzyme variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics 70: 113-139.

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

Bell, M. A. 1976. Evolution of phenotypic diversity in Gasterosteus aculeatus on the Pacific coast of North America. Syst. Zool. 25: 211-227.

Bell, M. A. and T. R. Haglund. 1978. Selective predation of threespine sticklebacks (Gasterosteus aculeatus) by garter snakes. Evolution 32: 304-319.

Berger, E. M. 1971. A temporal survey of allelic variation in natural and laboratory populations of D. melanogaster. Genetics 67: 121-136.

Chilson, O. P., G. B. Kitto, J. Pudles and N. O. Kaplan. 1966. Reversible inactivation of dehydrogenases. J. Biol. Chem. 241: 2431-2445.

Christiansen, F. B., O. Frydenberg, A. O. Gyldenholm and V. Simonsen. 1974. Genetics of Zoarces populations. VI. Further evidence, based on age group samples, of a heterozygote deficit in the Est-III polymorphism. Hereditas 77: 225-236.

Christiansen, F. B., O. Frydenberg and V. Simonsen. 1973. Genetics of Zoarces populations. IV. Selection component

analysis of an esterase polymorphism using population samples including mother-offspring combinations. Hereditas 73: 291-304.

Clayton, J. W., R. E. K. Harris and D. N. Tretiak. 1973. Identification of supernatant and mitochondrial isozymes of malate dehydrogenase on electropherograms applied to the taxonomic discrimination of walleye (Stizostedion vitreum vitreum), sauger (S. canadense), and suspected interspecific hybrid fishes. J. Fish. Res. Board. Can. 30: 927-938.

Clayton, J. W. and D. N. Tretiak. 1972. Amine citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board 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. Board Can. 28: 1005-1008.

Davidson, R. G. and J. A. Cortner. 1967. Mitochondrial malate dehydrogenase: a new genetic polymorphism in man. Science 157: 1569-1570.

Dawson, D. M., H. M. Eppenberger and N. O. Kaplan. 1967. The comparative enzymology of creatine kinases. II. Physical and chemical properties. J. Biol. Chem. 242: 210-217.

Dawson, D. M. and J. M. Greene. 1975. Phosphoglucomutase isozymes caused by sulfhydryl oxidation. In: Isozymes I. Molecular Structure (C. L. Markert, ed.), pp. 381-388. Academic Press.

Dobzhansky, Th. and F. J. Ayala. 1973. Temporal frequency

changes of enzyme and chromosomal polymorphisms in natural populations of Drosophila. Proc. Nat. Acad. Sci. U.S.A. 70: 680-683.

Engel, W., J. Faust and U. Wolf. 1971. Isoenzyme polymorphism of the sorbitol dehydrogenase and the NADP-dependent isocitrate dehydrogenases in the fish family Cyprinidae. Anim. Blood Grps. Biochem. Genet. 2: 127-133.

Ferris, S. D. and G. S. Whitt. 1978. Genetic and molecular analysis of nonrandom dimer assembly of the creatine kinase isozymes of fishes. Biochem. Genet. 16: 811-829.

Frydenberg, O., A. O. Gyldenholm, J. P. Hjorth and V. Simonsen. 1973. Genetics of Zoarces populations. III. Geographic variations in the esterase polymorphism Est-III. Hereditas 73: 233-238.

Fukunaga, A., A. Tanaka and K. Oishi. 1975. Maleless, a recessive autosomal mutant of Drosophila melanogaster that specifically kills male zygotes. Genetics 81: 135-141.

Gaines, M. S., L. R. McClenghan Jr. and R. K. Rose. 1978. Temporal patterns of allozymic variation in fluctuating populations of Microtus ochrogaster. Evolution 32: 723-739.

Gorman, G. C., M. Soule, S. Y. Yang and E. Nevo. 1975. Evolutionary genetics of insular adriatic lizards. Evolution 29: 52-71.

Gosselin-Rey, C., G. Hamoir and R. K. Scopes. 1968. Localization of creatine kinase in the starch-gel and moving-boundary electrophoretic patterns of fish muscle. J. Fish. Res. Board Can. 25: 2711-2714.

Gosselin-Rey, C. and C. Gerday. 1970. Isolation and molecular

properties of creatine kinase from carp white muscle. Biochim. Biophys. Acta 221: 241-254.

Gracy, R. W. 1975. Nature of the multiple forms of glucosephosphate and triosephosphate isomerases. In: Isozymes I. Molecular Structure (C. L. Markert, ed.), pp. 471-487. Academic Press.

Gross, H. P. 1978. Natural selection by predators on the defensive apparatus of of the three-spined stickleback, Gasterosteus aculeatus L. @ can. J. Zool. 56: 398-413.

Hagen, D. W. 1967. Isolating mechanisms in threespine sticklebacks (Gasterosteus). J. Fish. Res. Board Can. 24: 1637-1692.

Hagen, D. W. 1973. Inheritance of numbers of lateral plates and gill rakers in Gasterosteus aculeatus. Heredity 30: 303-312.

Hagen, D. W. and L. G. Gilbertson. 1972. Geographic variation and environmental selection in Gasterosteus aculeatus in the Pacific Northwest, America. Evolution 26: 32-51.

Hagen, D. W. and L. G. Gilbertson. 1973a. The genetics of plate morphs in freshwater threespine sticklebacks. Heredity 31: 75-84.

Hagen, D. W. and L. G. Gilbertson. 1973b. Selective predation and the intensity of selection acting upon the lateral plates of threespine sticklebacks. Heredity 30: 273-287.

Hagen, D. W. and J. D. McPhail. 1970. The species problem within Gasterosteus aculeatus on the Pacific coast of North America. J. Fish. Res Board Can. 27: 147-155.

Hamrick, J. L. and R. W. Allard. 1975. Correlations between

quantitative characters and enzyme genotypes in Avena barbata. Evolution 29: 438-442.

Hedgecock, D. 1978. Population subdivision and genetic divergence in the red-bellied newt, Taricha rivularis. Evolution 32: 271-286.

Henderson, N. S. 1968. Intracellular location and genetic control of isozymes of NADP-dependent isocitrate dehydrogenase and malate dehydrogenase. Ann. N.Y. Acad. Sci. 151, Art. 1: 429-440.

Henricson, J. and L. Nyman. 1976. The ecological and genetical segregation of two sympatric species of dwarfed char (Salvelinus alpinus (L.) species complex). Inst. Freshwater Res. Drottningholm Rep. 55: 15-37.

Heuts, M. J. 1947. Experimental studies on adaptive evolution in Gasterosteus aculeatus L. Evolution 1: 89-102.

International Union of Biochemistry, Standing Committee on Enzymes. 1965. Enzyme Nomenclature. Elsevier Sci. Pub. Co. Amsterdam.

Johnson, M. S. 1976. Allozymes and area effects in Cepea memoralis on the western Berkshire Downs. Heredity 36: 105-121.

Johnson, M. S. 1971. Adaptive lactate dehydrogenase variation in the crested blenny, Anoplarchus. Heredity 27: 205-226.

Joshi, J. G., J. Hooper, T. Kuwaki, T. Sakurada. J. R. Swanson and P. Handler. 1967. Phosphoglucomutase, V. Multiple forms of phosphoglucomutase. Proc. Nat. Acad. Sci. U. S. A. 57: 1482-1489.

Kirkpatrick, M. and R. K. Selander. 1979. Genetics of speciation

in lake whitefishes in the Allegash Basin. Evolution 33: 478-485.

Kitto, G. B. and R. G. Lewis. 1967. Purification and properties of tuna supernatant and mitochondrial malate dehydrogenases. Biochim. Biophys. Acta 139: 1-15.

Kitto, G. B., P. M. Wassarman, J. Michjeda and N. O. Kaplan. 1966. Multiple forms of mitochondrial malate dehydrogenases. Biochem. Biophys. Res. Commun. 22: 75-81.

Koehn, R. 1969. Esterase heterogeneity: Dynamics of a polymorphism. Science 163: 943-944.

Koehn, R. K. and D. I. Rasmussen. 1967. Polymorphic and monomorphic serum esterase heterogeneity in catostomid fish populations. Biochem. Genet. 1: 131-144.

Komma, D. J. 1968. Glucose-6-phosphate dehydrogenase in Drosophila: a sex-influenced electrophoretic variant. Biochem. Genet. 1: 229-237.

Kynard, B. E. 1972. Male breeding behavior and lateral plate phenotypes in the threespine stickleback (Gasterosteus aculeatus L.). Diss. Abstr. Sec. B 33: 2413-B.

Larsen, G. L. 1976. Social behavior and feeding ability of two phenotypes of Gasterosteus aculeatus in relation to their spatial and trophic segregation in a temperate lake. Can. J. Zool. 54: 107-121.

Lush, I. E. 1969 polymorphism of a phosphoglucomutase isoenzyme in the herring (Clupea harengus). Comp. Biochem. Physiol. 30: 391-395.

Manzer, J. I. 1976. Distribution, food and feeding of the threespine stickleback, Gasterosteus aculeatus, in Great

Central Lake, Vancouver Island, with comments on competition for food with juvenile sockeye salmon, Oncorhynchus nerka. Fishery Bull. 74: 647-668. Pac. Biol. Station, Dept. Of Environment, Canada.

Markert, C. L., J. B. Shaklee and G. S. Whitt. 1975. Evolution of a gene. Science 189: 102-114.

Mathews, W. H., J. G. Fyles and H. W. Nasmith. 1970. Postglacial crustal movements in southwestern British Columbia and adjacent Washington state. Can. J. Earth Sci. 7: 690-702.

May, B. 1975. Electrophoretic variation in the genus Oncorhynchus: the methodology, genetic basis and practical applications to fisheries research and management. M.Sc. Thesis, Univ. of Washington, Seattle.

McPhail, J. D. 1969. Predation and the evolution of a stickleback (Gasterosteus). J. Fish. Res. Board Can. 26: 3183-3208.

McPhail, J. D. and C. C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173. 381 pp.

Merritt, R. B. 1972. Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, Pimephales promelas. Amer. Nat. 196: 173-184.

Merritt, R. B., J. F. Rogers and B. Kurz. 1978. Genic variability in the longnose dace, Rhinichthys cataractae. Evolution 32: 116-124.

Michiel, G. P. 1977. An examination of two reported protein polymorphisms in threespine stickleback (Gasterosteus

aculeatus). M.Sc. Thesis, Dept. Zoology, Univ. of British Columbia, Vancouver.

Miller, R. R. and C. L. Hubbs. 1969. Systematics of Gasterosteus aculeatus, with particular reference to intergradation and introgression along the Pacific coast of North America: a commentary on a recent contribution. Copeia 1969: 52-69.

Mitton, J. B. and R. K. Koehn. 1975. Genetic organization and adaptive response of allozymes to ecological variables in Fundulus heteroclitus. Genetics 79: 97-111.

Moodie, G. E. E. 1972a. Morphology, life history and ecology of an unusual stickleback (Gasterosteus aculeatus) in the Queen Charlotte Islands, Canada. Can. J. Zool. 50: 721-732.

Moodie, G. E. E. 1972b. Predation, natural selection and adaptation in an unusual threespine stickleback. Heredity 28: 155-167.

Moodie, G. E. E., J. D. McPhail and D. W. Hagen. 1973. Experimental demonstration of selective predation in Gasterosteus aculeatus. Behaviour 47: 95-105.

Moodie, G. E. E. and T. E. Reimchen. 1976. Phenetic variation and habitat differences in Gasterosteus populations of the Queen Charlotte Islands. Syst. Zool. 25: 49-61.

Narver, D. W. 1969. Phenotypic variation in threespine sticklebacks (Gasterosteus aculeatus) of the Chignik River system, Alaska. J. Fish. Res. Board Can. 26: 405-412.

Nei, M. 1972. Genetic distance between populations. Amer. Nat. 106: 283-292.

Nei, M. 1975. A new measure of genetic distance. In: Genetic Distance (J. F. Crow and C. Denniston, eds.), pp. 63-76.

Plenum Press, New York.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. 13: 121-177.

Nilsson, N.-A. and O. Filipsson. 1971. Characteristics of two discrete populations of Arctic char (Salvelinus alpinus L.) in a north Swedish Lale. Inst. Freshwater Res. Drottningholm Rep. 51: 90-108.

Noltman, E. A. 1975. The different origins of multiple molecular forms of phosphoglucose isomerase. In: Isozymes I. Molecular Structure (C. L. Markert, ed.), pp. 451-470. Academic Press.

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.

Nyman, L. 1972. A new approach to the taxonomy of the "Salvelinus alpinus species complex." Inst. Freshwater Res. Drottningholm Rep 52: 103-131.

Nyman, L. 1975. Allelic selection in a fish (Gymnocephalus cernua (L.)) subjected to hotwater effluents. Inst. Freshwater Res. Drottningholm. Rep. No. 54: 75-82.

Quiroz-Gutierrez, A. and S. Ohno. 1970. The evidence of gene duplication for S-form NADP-linked isocitrate dehydrogenase in carp and goldfish. Biochem. Genet. 4: 93-99.

Raunich, L., C. Callegarini and C. Cucchi. 1972. Ecological aspects of hemoglobin polymorphism in Gasterosteus aculeatus (Teleostea). In: Fifth European symposium of marine biology, pp. 153-162. Piccin Publishers.

Reinitz, G. L. 1977. Inheritance of muscle and liver types of

supernatant NADP-dependent isocitrate dehydrogenase in rainbow trout (Salmo gairdneri). Biochem. Genet. 15: 445-454.

Ridgway, G. J., S. W. Sherburne and R. D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99: 147-151.

Roberts, F. L., J. F. Wohnus and S. Ohno. 1969. Phosphoglucomutase polymorphism in the rainbow trout, Salmo gairdneri. Experientia 25: 1109-1110.

Ross, S. T. 1973. The systematics of Gasterosteus aculeatus (Pisces: Gasterosteidae) in central and southern California. Natur. Hist. Mus. Los Angeles Co. Contrib. Sci. 243: 1-20.

Scopes, R. K. and C. Gosselin-Rey. 1968. Polymorphism in carp muscle creatine kinase. J. Fish. Res. Board Can. 25: 2715-2716.

Shaklee, J. B., M. J. Champion and G. S. Whitt. 1974. Developmental genetics of teleosts: a biochemical analysis of lake chubsucker ontogeny. Devel. Biol. 38: 356-382.

Shaw, C. R. 1965. Electrophoretic variation in enzymes. Science 149: 936-943.

Shaw, C. R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes: a compilation of recipes. Biochem. Genet. 4: 297-320.

Sick, K. 1965. Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. Hereditas 54: 19-48.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. Freeman, San Francisco. 776 pp.

Soule, M. and S. Y. Yang. 1973. Genetic variation in side-blotched lizards on islands in the Gulf of California. Evolution 27: 593-600.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures in Statistics. McGraw-Hill, New York. 481 pp.

Tanaka, A., A. Fukunaga and K. Oishi. 1976. Studies on the sex-specific lethals of Drosophila melanogaster. II. Further studies on a male-specific lethal gene, maleless. Genetics 84: 257-266.

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

Tomaszewski, E. K., H. E. Schaffer and F. M. Johnson. 1973. Isozyme genotype-environment associations in natural populations of the harvester ant, Pogonomyrmex badius. Genetics 75: 405-421.

Turner, B. M., R. A. Fisher and H. Harris. 1975. Post-translational alterations of human erythrocyte enzymes. In: Isozymes I. Molecular Structure (C. L. Markert, ed.), pp. 781-795. Academic press.

Utter, F. M. and H. O. Hodgins. 1970. Phosphoglucomutase polymorphism in sockeye salmon. Comp. Biochem. Physiol. 36: 195-199.

Ward, R. H. and C. F. Sing. 1970. A consideration of the power of the chi-square test to detect inbreeding effects in natural populations. Amer. Natur. 104: 355-365.

Wheat, T. E. and G. S. Whitt. 1971. In vivo and in vitro molecular hybridization of malate dehydrogenase isozymes.

Experientia 27: 647-648.

Whitt, G. S. 1970. Genetic variation of supernatant and mitochondrial malate dehydrogenase isozymes in the teleost Fundulus heteroclitus. Experientia 26: 734-736.

Wootton, R. J. 1976. The Biology of the Sticklebacks. Academic Press, London. 387 pp.

Workman, P. L. 1969. The analysis of simple genetic polymorphisms. Human Biol. 41: 97-114.

APPENDIX I

Locations and dates of *G. aculeatus* sample collections.

1. Marine Populations

Englishman River, estuary, Vancouver Island (ENGLR). June 1977. 49°20' 124°17'
Horseshoe Bay, west of Vancouver (HORSB). Nov. 1977. 49°23' 123°16'
Metchosin Lagoon, Sooke, Vancouver Island (WITLG). June 1977. 48°23' 123°31'
Little Campbell River, estuary, White Rock, B.C. (LC 19). June 1978. 49°01' 122°46'
Cowichan Bay, Vancouver Island (COWIB). June 1977. 48°44' 123°38'
Pender Harbour, Madeira Park Wharf, Sechelt Peninsula (MDERA). April 1978. 49°37' 124°01'
Sooke River, Vancouver Island (SOOKP). June 1977. 48°24' 123°42'
Koksilah River, estuary, Vancouver Island (KOKSR). June 1977. 48°46' 123°40'
Stream entering Ucluelet Inlet, 3 km N of Thornton Creek, Vancouver Island (SLMNC). July 1978. 48°59' 125°34'
Grappler Inlet, Vancouver Island (GRAPI). May 1978. 48°50' 125°07'
Small stream near Bamfield, Vancouver Island (BAMFS). Aug. 1978. 48°52' 125°06'
Sarita River, estuary, Vancouver Island (SARIE). May 1978. 48°54' 125°01'
Haines Island, lagoon, W of Vancouver Island (HAINL). May 1978. 48°50' 125°12'
Congreve Island, W of Vancouver Island (CONGR). May 1978. 48°56' 125°02'
Santa Maria Island, W of Vancouver Island (SANMA). May 1978. 48°53' 125°01'
Chehalis River, at Wensall Rd., Wash. State (CHEHW). June 1978. 46°59' 123°22'

2. Large Lake Populations

Cowichan Lake, Vancouver Island (COWIL). Sept. 1977. 48°50' 124°12'
Sarita Lake, Vancouver Island (SARIL). May 1978. 48°55' 124°52'
Sakinaw Lake, Sechelt Peninsula (SAKIN). May 1978. 49°39' 124°03'
Sproat Lake, Vancouver Island (SPROT). May and Sept. 1978. 49°18' 124°56'
McCreight Lake, Vancouver Island (MCRTL). June and Sept. 1978. 50°17' 125°39'
Great Central Lake, Vancouver Island (GRCEN). May 1978. 49°19' 124°59'
Stella Lake, Vancouver Island (STELL). June 1978. 50°17' 125°30'
Roberts Lake, Vancouver Island (ROBTL). June 1978. 50°14' 125°32'

3. Small Lake Populations

Chemainus Lake, Vancouver Island (CHEML). Sept. 1977 and Oct. 1978. 48°55' 123°45'
Fuller Lake, Vancouver Island (FULLL). Sept. 1977. 48°54' 123°43'
Jacobs (Marion) Lake, Haney, B.C. (MARNL). Aug. 1977 and June 1979. 49°19' 122°33'
Lake Erroch (Squakum Lake), B.C. (LKERR). Aug. 1978. 49°14' 122°01'
Hotel Lake, Sechelt Peninsula (HOTEL). April 1978. 49°38' 124°03'
Klein Lake, Sechelt Peninsula (KLEIN). May 1978. 49°44' 124°03'
Pag Lake, Sechelt Peninsula (PAQLK). April 1978. 49°37' 124°02'
Trout Lake, Sechelt Peninsula (TROUT). April 1978. 49°31' 123°53'
Garden Bay Lake, Sechelt Peninsula (GARBY). May 1978. 49°38' 124°02'
Blackwater Lake, Vancouver Island (BLACK). June and Sept. 1978. 50°11' 125°35'
Patterson Lake, Vancouver Island (PATER). May and Sept. 1978 49°21' 125°00'
Devil's Den Lake, Vancouver Island (DEVIL). May 1978. 49°15' 124°52'
Sumner Lake, Vancouver Island (SUMNL). May and Sept. 1978. 49°22' 124°59'
Mud Lake, Vancouver Island (MUDLK). June and Sept. 1978. 50°12' 125°33'
Lowry Lake, Vancouver Island (LOWRL). May and Sept. 1978. 49°24' 125°08'
McCoy Lake, Vancouver Island (MCOYL). May and Sept. 1978. 49°16' 124°53'
Cecil Lake, Vancouver Island (CECIL). June and Sept. 1978. 50°14' 125°33'
Morgan Lake, Vancouver Island (MORGL). June and Sept. 1978. 50°13' 125°33'
Ormond Lake, Vancouver Island (ORMND). June 1978. 50°11' 125°31'
Farewell Lake, Vancouver Island (FARWL). June and Sept. 1978. 50°12' 125°35'
Cedar Lake, Vancouver Island (CEDAR). June and Sept. 1978. 50°12' 125°34'
Paxton Lake, Texada Island (PAXTB and PAXTL). Oct. 1977. 49°43' 124°31'
Enos Lake, Vancouver Island (ENOSB and ENOSL). Oct. 1977 and Sept. 1978. 49°17' 124°09'
Goose Lake, Haney B.C. (GOOSE). Nov. 1977. 49°18' 122°36'
Hiechhold Lake, Texada Island (HECHL). Oct. 1977. 49°46' 124°35'
Cranby Lake, Texada Island (CRANL). Oct. 1977. 49°42' 124°30'

4. Low-lying Stream and Swamp Populations

Tugwell Creek, Sooke, Vancouver Island (SKOOS). June 1977. 48°22' 123°51'
Slough N of Wilfred Creek, Ship Peninsula, Vancouver Island (SHIPC). June 1977. 49°30' 124°48'
Little Campbell River, 15 km upstream from mouth, White Rock,

B.C. (LC 18). March and June 1978. 49°02' 122°39'
Gifford Slough, McLennan Creek, Fraser River Valley (GIFFS). May 1977. 49°07' 122°20'
Yorkson Creek, Fraser River Valley (TEXAS). May 1977. 49°12' 122°39'
Stream entering Deep Cove, Saanich Inlet, Vancouver Island (PATBS). June 1977. 48°39' 123°27'
Snohomish River, slough near Ebey Island, Wash. State (EBEYI). June 1978. 48°01' 122°09'
North Skagit River, Wash. State (SKAGI). June 1978. 48°21' 122°27'
Slough on Westham Island, S arm Fraser River estuary (MOUSE). May 1978. 49°06' 123°10'
Roadside swamp 3 km NE of McCreight Lake, Vancouver Island (SMUDL). June 1978. 50°19' 125°36'
Nathan Creek, Fraser River Valley (NATHN). May 1977. 49°08' 122°28'
Colquitz River, Vancouver Island (CLQTZ). Sept. 1977. 48°28' 123°24'
Roadside pond 2 km S of McCreight Lake, Vancouver Island (MCRPD). June 1978. 50°16' 125°39'
Sarita River, junction of N and S arms, Vancouver Island (SARIJ). 48°54' 124°59'
Serpentine River, pond near Bothwell Park, Surrey, B.C. (TYNEP). March, June and Aug. 1978. June 1979. 49°10' 122°45'
Chehalis River, at Hoquiam Beach Road, Wash. State (HOQUB). Oct. 1978. 47°00' 123°53'
Roadside slough 2 km E of Otter Point, Sooke, Vancouver Island (OTTER). June 1977. 48°22' 123°48'
Swamp between Blackwater and Farewell lakes, Vancouver Island (FARBL). June and Sept. 1978. 50°11' 125°35'

5. Isolated Stream Populations

Keogh River, 30 km upstream from mouth, Vancouver Island (KEOGH). May 1977. 50°32' 127°13'
Roadside swamp N of Farewell Lake, Vancouver Island (FARSW). June 1978. 50°13' 125°35'
Roadside pond NW of Taylor Arm, Sproat Lake, Vancouver Island (SPRPD). May and Sept. 1978. 49°17' 125°14'
Salzer River, near Centralia, Wash. State (SLZER). June 1978. 46°42' 122°57'
Dry Run Creek, Chehalis River drainage, Wash. State (DRYRN). June 1978. 47°08' 123°20'

6. Mixed Populations

Fraser River, S arm near Woodward Island (FRASR). May 1978. 49°07' 123°10'
Lard Creek, Vancouver Island (LARDC). June 1978. 49°40' 124°58'
Fuller Creek, Vancouver Island (FULCR). June 1977. 48°55' 123°42'
Little Campbell River, 2.5 km upstream from mouth, White Rock, B.C. (LC 40). June 1978. 49°01' 122°45'

APPENDIX II

Allele frequency distribution at the Mdh-3 locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Mdh-3^{100}	Mdh-3^{55}	Heterozygotes Observed (Exp)	Hetero-zygosity
Marine					
ENGLR	30	0.000	1.000	0 (0)	0.000
HORSB	--	---	---	---	---
WITLG	24	0.125	0.875	2 (5)	0.219
LC 19	57	0.430	0.570	31 (28)	0.490
COWIB	27	0.056	0.944	3 (3)	0.106
MDERA	30	0.117	0.883	7 (6)	0.207
SOOKP	31	0.097	0.903	4 (5)	0.175
KOKSR	69	0.188	0.812	20 (21)	0.305
SLMNC	53	0.160	0.840	11 (14)	0.269
GRAPI	41	0.183	0.817	13 (12)	0.299
BAMFS	34	0.176	0.824	6 (10)	0.290
SARIE	44	0.159	0.841	14 (12)	0.267
HAINL	23	0.217	0.783	4 (8)	0.340
CONGR	42	0.095	0.905	6 (7)	0.172
SANMA	49	0.112	0.888	9 (10)	0.199
CHEHW	16	0.094	0.906	3 (3)	0.170
Large Lake					
COWIL	37	0.000	1.000	0 (0)	0.000
SARIL	57	0.816	0.184	9 (17) *	0.300
SAKIN	11	0.364	0.636	8 (5)	0.463
SPROT	137	0.588	0.412	59 (66)	0.485
MCRTL	258	0.764	0.236	96 (93)	0.361
GRCEN	53	0.462	0.538	21 (26)	0.497
STELL	19	0.895	0.105	4 (4)	0.188
ROBTL	21	0.524	0.476	12 (10)	0.499
Small Lake					
CHEML	83	1.000	0.000	0 (0)	0.000
FULLL	32	0.641	0.359	15 (15)	0.460
MARNL	75	1.000	0.000	0 (0)	0.000
LKERR	46	0.359	0.641	27 (21)	0.460
HOTEL	114	0.570	0.430	52 (56)	0.490
KLEIN	45	1.000	0.000	0 (0)	0.000
PAQLK	41	0.976	0.024	2 (2)	0.047
TROUT	50	0.840	0.160	16 (13)	0.269
GARBY	14	0.964	0.036	1 (1)	0.069
BLACK	250	0.500	0.500	118 (125)	0.500
PATER	106	0.962	0.038	8 (8)	0.073
DEVIL	84	0.464	0.536	46 (42)	0.497
SUMNL	66	0.992	0.008	1 (1)	0.016
MUDLK	59	0.000	1.000	0 (0)	0.000

LOWRL	47	0.298	0.702	18 (20)	0.418
MCOYL	129	0.926	0.074	15 (18)	0.137
CECIL	103	0.267	0.733	45 (40)	0.391
MORGL	82	0.000	1.000	0 (0)	0.000
ORMND	62	0.040	0.960	5 (5)	0.077
FARWL	139	0.500	0.500	79 (70)	0.500
CEDAR	108	0.213	0.787	38 (36)	0.335
PAXTB	112	0.987	0.013	3 (3)	0.026
PAXTL	41	0.707	0.293	18 (17)	0.414
ENOSB	124	1.000	0.000	0 (0)	0.000
ENOSL	152	0.822	0.178	38 (44)	0.293
GOOSE	63	0.603	0.397	34 (30)	0.479
HECHL	28	0.643	0.357	10 (13)	0.459
CRANL	48	1.000	0.000	0 (0)	0.000
Low-lying Stream or Swamp					
SKOOS	--	---	---	---	---
SHIPC	27	0.130	0.870	5 (6)	0.226
LC 18	87	0.805	0.195	32 (27)	0.314
GIFFS	13	0.923	0.077	2 (2)	0.142
TEXAS	19	0.684	0.316	12 (8)	0.432
PATBS	49	1.000	0.000	0 (0)	0.000
EBEYI	68	0.507	0.493	25 (34) *	0.500
SKAGI	37	0.541	0.459	16 (18)	0.497
MOUSE	36	0.347	0.653	15 (16)	0.453
SMUDL	78	1.000	0.000	0 (0)	0.000
NATHN	39	0.295	0.705	17 (16)	0.416
CLQTZ	47	0.766	0.234	16 (17)	0.358
MCRPD	98	0.786	0.214	38 (33)	0.336
SARIJ	39	0.603	0.397	13 (19)	0.479
TYNEP	322	0.138	0.862	53 (77) **	0.238
HOQUB	33	0.030	0.970	0 (2)	0.058
OTTER	117	0.295	0.705	49 (49)	0.416
FARBL	172	0.506	0.494	84 (86)	0.500
Isolated Stream or Swamp					
KEOGH	45	1.000	0.000	0 (0)	0.000
FARSW	74	0.372	0.628	25 (35) *	0.467
SPRPD	166	0.726	0.274	59 (66)	0.398
SLZER	61	0.189	0.811	13 (19)	0.307
DRYRN	50	0.530	0.470	23 (25)	0.498
Mixed					
FRASR	100	0.390	0.610	46 (48)	0.476
LARDC	85	0.729	0.271	36 (34)	0.395
FULCR	63	0.230	0.770	19 (22)	0.354
LC 40	43	0.419	0.581	16 (21)	0.487

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* $P < 0.05$

** $P < 0.01$

APPENDIX III

Allele frequency distribution at the Mdh-1 locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Mdh-1^{100}	Mdh-1^{82}	Other	Heterozygotes Observed	(Exp)	Hetero-zygosity
Marine							
ENGLR	29	0.914	0.086	---	5	(5)	0.157
HORSB	30	0.967	0.033	---	2	(2)	0.064
WITLG	24	1.000	0.000	---	0	(0)	0.000
LC 19	57	0.974	0.026	---	3	(3)	0.051
COWIB	27	1.000	0.000	---	0	(0)	0.000
MDERA	16	0.969	0.031	---	1	(1)	0.060
SOOKP	32	0.922	0.078	---	5	(5)	0.144
KOKSR	65	0.977	0.023	---	3	(3)	0.045
SLMNC	53	0.991	0.009	---	1	(1)	0.018
GRAPI	41	0.988	0.012	---	1	(1)	0.024
BAMFS	34	0.912	0.088	---	6	(5)	0.161
SARIE	44	0.909	0.091	---	6	(7)	0.165
HAINL	23	1.000	0.000	---	0	(0)	0.000
CONGR	36	0.944	0.056	---	4	(4)	0.106
SANMA	44	0.943	0.057	---	5	(5)	0.108
CHEHW	16	0.844	0.156	---	5	(4)	0.263
Large Lake							
COWIL	37	0.946	0.054	---	4	(4)	0.102
SARIL	57	0.658	0.342	---	29	(26)	0.450
SAKIN	11	0.818	0.182	---	4	(3)	0.298
SPROT	138	0.862	0.138	---	30	(33)	0.238
MCRTL	260	0.856	0.144	---	61	(64)	0.247
GRCEN	53	0.981	0.019	---	2	(2)	0.037
STELL	19	0.842	0.158	---	6	(5)	0.266
ROBTL	21	1.000	0.000	---	0	(0)	0.000
Small Lake							
CHEML	81	0.975	0.025	---	4	(4)	0.049
FULLL	37	0.730	0.270	---	16	(15)	0.394
MARNL	75	0.933	0.067	---	10	(9)	0.125
LKERR	46	0.967	0.033	---	3	(3)	0.064
HOTEL	114	0.987	0.013	---	3	(3)	0.026
KLEIN	45	0.711	0.289	---	20	(18)	0.411
PAQLK	41	1.000	0.000	---	0	(0)	0.000
TROUT	50	1.000	0.000	---	0	(0)	0.000
GARBY	14	1.000	0.000	---	0	(0)	0.000
BLACK	256	0.984	0.016	---	8	(8)	0.031
PATER	107	1.000	0.000	---	0	(0)	0.000
DEVIL	85	1.000	0.000	---	0	(0)	0.000
SUMNL	66	0.992	0.008	---	1	(1)	0.016
MUDLK	59	1.000	0.000	---	0	(0)	0.000

LOWRL	47	0.989	0.011	---	1	(1)	0.022
MCOYL	130	0.988	0.012	---	3	(3)	0.024
CECIL	103	1.000	0.000	---	0	(0)	0.000
MORGL	82	1.000	0.000	---	0	(0)	0.000
ORMND	62	1.000	0.000	---	0	(0)	0.000
FARWL	139	0.950	0.050	---	14	(13)	0.095
CEDAR	108	1.000	0.000	---	0	(0)	0.000
PAXTB	113	0.965	0.035	---	6	(8)	0.068
PAXTL	44	0.977	0.023	---	2	(2)	0.045
ENOSB	125	1.000	0.000	---	0	(0)	0.000
ENOSL	154	1.000	0.000	---	0	(0)	0.000
GOOSE	66	1.000	0.000	---	0	(0)	0.000
HECHL	37	1.000	0.000	---	0	(0)	0.000
CRANL	48	1.000	0.000	---	0	(0)	0.000
Low-lying Stream or Swamp							
SKOOS	35	0.957	0.043	---	3	(3)	0.082
SHIPC	27	1.000	0.000	---	0	(0)	0.000
LC 18	87	0.983	0.017	---	3	(3)	0.033
GIFFS	13	0.923	0.077	---	2	(2)	0.142
TEXAS	26	0.942	0.058	---	3	(3)	0.109
PATBS	49	0.510	0.490	---	20	(24)	0.500
EBEYI	68	0.831	0.154	0.015	19	(19)	0.286
SKAGI	37	0.946	0.054	---	4	(4)	0.102
MOUSE	36	0.903	0.803	0.014	5	(6)	0.178
SMUDL	78	0.583	0.417	---	39	(38)	0.486
NATHN	41	0.854	0.146	---	8	(10)	0.249
CLQTZ	47	1.000	0.000	---	0	(0)	0.000
MCRPD	100	0.875	0.125	---	19	(22)	0.219
SARIJ	39	0.897	0.103	---	8	(7)	0.185
TYNEP	323	1.000	0.000	---	0	(0)	0.000
HOQUB	33	0.985	0.015	---	1	(1)	0.030
OTTER	117	0.996	0.004	---	1	(1)	0.008
FARBL	172	0.953	0.047	---	16	(15)	0.090
Isolated Stream or Swamp							
KEOGH	45	1.000	0.000	---	0	(0)	0.000
FARSW	75	0.920	0.080	---	12	(11)	0.147
SPRPD	166	0.976	0.024	---	8	(8)	0.047
SLZER	61	0.402	0.000	0.598	25	(29)	0.481
DRYRN	50	1.000	0.000	---	0	(0)	0.000
Mixed							
FRASR	106	0.976	0.024	---	5	(5)	0.047
LARDC	88	0.983	0.017	---	3	(3)	0.033
FULCR	63	0.968	0.032	---	4	(4)	0.062
LC 40	45	0.967	0.033	---	3	(3)	0.064

APPENDIX IV

Allele frequency distribution at the Pgi-1 locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Pgi-1^{100}	Pgi-1^{105}	Other	Heterozygotes Observed	(Exp)	Hetero-zygosity
Marine							
ENGLR	30	0.950	0.033	0.017	1	(3)	0.096
HORSB	37	1.000	0.000	---	0	(0)	0.000
WITLG	28	0.964	0.036	---	0	(2)	0.069
LC 19	57	1.000	0.000	---	0	(0)	0.000
COWIB	27	1.000	0.000	---	0	(0)	0.000
MDERA	18	1.000	0.000	---	0	(0)	0.000
SOOKP	33	0.985	0.015	---	1	(1)	0.030
KOKSR	64	1.000	0.000	---	0	(0)	0.000
SLMNC	53	0.962	0.038	---	0	(4)	0.073
GRAPI	41	0.976	0.024	---	0	(2)	0.047
BAMFS	34	0.971	0.029	---	0	(2)	0.056
SARIE	44	1.000	0.000	---	0	(0)	0.000
HAINL	23	1.000	0.000	---	0	(0)	0.000
CONGR	35	0.971	0.029	---	0	(2)	0.056
SANMA	30	1.000	0.000	---	0	(0)	0.000
CHEHW	16	1.000	0.000	---	0	(0)	0.000
Large Lake							
COWIL	37	0.973	0.027	---	0	(2)	0.053
SARIL	57	1.000	0.000	---	0	(0)	0.000
SAKIN	11	0.955	0.045	---	1	(1)	0.086
SPROT	138	1.000	0.000	---	0	(0)	0.000
MCRTL	218	1.000	0.000	---	0	(0)	0.000
GRCEN	53	0.821	0.179	---	11	(16)	0.294
STELL	18	0.972	0.000	0.028	1	(1)	0.054
ROBTL	21	1.000	0.000	---	0	(0)	0.000
Small Lake							
CHEML	79	1.000	0.000	---	0	(0)	0.000
FULLL	37	1.000	0.000	---	0	(0)	0.000
MARNL	75	1.000	0.000	---	0	(0)	0.000
LKERR	46	1.000	0.000	---	0	(0)	0.000
HOTEL	114	0.996	0.000	0.004	1	(1)	0.008
KLEIN	45	0.978	0.022	---	0	(2)	0.043
PAQLK	41	1.000	0.000	---	0	(0)	0.000
TROUT	50	1.000	0.000	---	0	(0)	0.000
GARBY	14	1.000	0.000	---	0	(0)	0.000
BLACK	170	0.994	0.003	0.003	2	(2)	0.012
PATER	107	0.991	0.009	---	0	(2)	0.018
DEVIL	85	1.000	0.000	---	0	(0)	0.000
SUMNL	66	1.000	0.000	---	0	(0)	0.000
MUDLK	59	1.000	0.000	---	0	(0)	0.000

LOWRL	30	1.000	0.000	---	0	(0)	0.000
MCOYL	129	1.000	0.000	---	0	(0)	0.000
CECIL	103	1.000	0.000	---	0	(0)	0.000
MORGL	81	1.000	0.000	---	0	(0)	0.000
ORMND	62	1.000	0.000	---	0	(0)	0.000
FARWL	139	1.000	0.000	---	0	(0)	0.000
CEDAR	107	0.991	0.009	---	0	(2)	0.018
PAXTB	113	1.000	0.000	---	0	(0)	0.000
PAXTL	44	0.977	0.023	---	0	(2)	0.045
ENOSB	125	1.000	0.000	---	0	(0)	0.000
ENOSL	154	1.000	0.000	---	0	(0)	0.000
GOOSE	67	1.000	0.000	---	0	(0)	0.000
HECHL	37	1.000	0.000	---	0	(0)	0.000
CRANL	48	1.000	0.000	---	0	(0)	0.000
Low-lying Stream or Swamp							
SKOOS	35	1.000	0.000	---	0	(0)	0.000
SHIPC	27	1.000	0.000	---	0	(0)	0.000
LC 18	50	1.000	0.000	---	0	(0)	0.000
GIFFS	13	1.000	0.000	---	0	(0)	0.000
TEXAS	26	1.000	0.000	---	0	(0)	0.000
PATBS	49	1.000	0.000	---	0	(0)	0.000
EBEYI	68	0.993	0.000	0.007	1	(1)	0.014
SKAGI	37	0.865	0.135	---	0	(9)	0.234
MOUSE	36	0.986	0.014	---	1	(1)	0.028
SMUDL	78	1.000	0.000	---	0	(0)	0.000
NATHN	--	---	---	---	---		---
CLQTZ	47	1.000	0.000	---	0	(0)	0.000
MCRPD	100	1.000	0.000	---	0	(0)	0.000
SARIJ	39	1.000	0.000	---	0	(0)	0.000
TYNEP	299	1.000	0.000	---	0	(0)	0.000
HOQUB	33	1.000	0.000	---	0	(0)	0.000
OTTER	117	1.000	0.000	---	0	(0)	0.000
FARBL	172	1.000	0.000	---	0	(0)	0.000
Isolated Stream or Swamp							
KEOGH	45	1.000	0.000	---	0	(0)	0.000
FARSW	75	1.000	0.000	---	0	(0)	0.000
SPRPD	168	1.000	0.000	---	0	(0)	0.000
SLZER	23	1.000	0.000	---	0	(0)	0.000
DRYRN	50	1.000	0.000	---	0	(0)	0.000
Mixed							
FRASR	106	0.995	0.005	---	1	(1)	0.010
LARDC	126	0.992	0.008	---	0	(2)	0.016
FULCR	63	1.000	0.000	---	0	(0)	0.000
LC 40	45	1.000	0.000	---	0	(0)	0.000

APPENDIX V

Allele frequency distribution at the Pgi-2 locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Pgi-2^{100}	Pgi-2^{147}	Other	Heterozygotes Observed	(Exp)	Hetero-zygosity
Marine							
ENGLR	30	0.983	0.017	---	1	(1)	0.033
HORSB	37	0.986	0.014	---	1	(1)	0.028
WITLG	28	1.000	0.000	---	0	(0)	0.000
LC 19	57	0.982	0.018	---	2	(2)	0.035
COWIB	27	0.981	0.019	---	1	(1)	0.037
MDERA	30	0.950	0.050	---	3	(3)	0.095
SOOKP	33	0.955	0.045	---	3	(3)	0.086
KOKSR	68	0.919	0.081	---	11	(10)	0.149
SLMNC	53	0.953	0.038	0.009	3	(5)	0.090
GRAPI	41	0.915	0.073	0.012	7	(6)	0.157
BAMFS	34	0.809	0.191	---	11	(11)	0.309
SARIE	43	0.907	0.047	0.047	8	(8)	0.173
HAINL	23	0.978	0.022	---	1	(1)	0.043
CONGR	44	0.909	0.091	---	8	(7)	0.165
SANMA	49	0.918	0.051	0.031	8	(8)	0.154
CHEHW	16	0.906	0.094	---	3	(3)	0.170
Large Lake							
COWIL	37	0.973	0.014	0.014	2	(2)	0.053
SARIL	57	0.939	0.061	---	7	(7)	0.115
SAKIN	11	0.955	0.045	---	1	(1)	0.086
SPROT	138	0.978	0.022	---	6	(6)	0.043
MCRTL	262	0.973	0.004	0.023	14	(14)	0.053
GRCEN	53	0.877	0.123	---	11	(11)	0.216
STELL	18	0.861	0.139	---	5	(4)	0.239
ROBTL	21	1.000	0.000	---	0	(0)	0.000
Small Lake							
CHEML	79	0.816	0.184	---	23	(24)	0.300
FULLL	35	0.457	0.514	0.029	22	(18)	0.526
MARNL	75	0.773	0.227	---	24	(26)	0.351
LKERR	46	0.978	0.022	---	2	(2)	0.043
HOTEL	114	0.978	0.022	---	5	(5)	0.043
KLEIN	45	0.989	0.011	---	1	(1)	0.022
PAQLK	41	1.000	0.000	---	0	(0)	0.000
TROUT	50	0.990	0.010	---	1	(1)	0.020
GARBY	14	1.000	0.000	---	0	(0)	0.000
BLACK	256	1.000	0.000	---	0	(0)	0.000
PATER	107	1.000	0.000	---	0	(0)	0.000
DEVIL	85	1.000	0.000	---	0	(0)	0.000
SUMNL	65	1.000	0.000	---	0	(0)	0.000
MUDLK	59	1.000	0.000	---	0	(0)	0.000

LOWRL	47	1.000	0.000	---	0	(0)	0.000
MCOYL	130	0.965	0.035	---	9	(9)	0.068
CECIL	103	0.820	0.180	---	27	(30)	0.295
MORGL	81	0.963	0.000	0.037	6	(6)	0.071
ORMND	62	1.000	0.000	---	0	(0)	0.000
FARWL	138	0.978	0.022	---	6	(6)	0.043
CEDAR	108	1.000	0.000	---	0	(0)	0.000
PAXTB	113	0.978	0.022	---	5	(5)	0.043
PAXTL	44	0.693	0.307	---	21	(19)	0.426
ENOSB	125	0.920	0.080	---	20	(18)	0.147
ENOSL	153	0.944	0.056	---	17	(16)	0.106
GOOSE	67	0.925	0.075	---	10	(9)	0.139
HECHL	37	0.986	0.014	---	1	(1)	0.028
CRANL	48	1.000	0.000	---	0	(0)	0.000
Low-lying Stream or Swamp							
SKOOS	35	1.000	0.000	---	0	(0)	0.000
SHIPC	27	0.981	0.019	---	1	(1)	0.037
LC 18	87	1.000	0.000	---	0	(0)	0.000
GIFFS	13	1.000	0.000	---	0	(0)	0.000
TEXAS	26	0.981	0.019	---	1	(1)	0.037
PATBS	49	0.929	0.071	---	7	(6)	0.132
EBEYI	68	0.897	0.103	---	14	(13)	0.185
SKAGI	37	0.973	0.027	---	2	(2)	0.053
MOUSE	36	0.931	0.042	0.028	5	(5)	0.131
SMUDL	78	1.000	0.000	---	0	(0)	0.000
NATHN	--	---	---	---	---		---
CLQTZ	47	0.702	0.277	0.021	22	(20)	0.430
MCRPD	99	0.970	0.010	0.020	6	(6)	0.059
SARIJ	39	1.000	0.000	---	0	(0)	0.000
TYNEP	302	0.967	0.033	---	20	(19)	0.075
HOQUB	33	1.000	0.000	---	0	(0)	0.000
OTTER	117	1.000	0.000	---	0	(0)	0.000
FARBL	172	0.977	0.023	---	8	(8)	0.045
Isolated Stream or Swamp							
KEOGH	45	1.000	0.000	---	0	(0)	0.000
FARSW	75	1.000	0.000	---	0	(0)	0.000
SPRPD	168	1.000	0.000	---	0	(0)	0.000
SLZER	23	1.000	0.000	---	0	(0)	0.000
DRYRN	50	1.000	0.000	---	0	(0)	0.000
Mixed							
FRASR	106	0.972	0.019	0.009	6	(6)	0.055
LARDC	126	0.948	0.048	0.004	11	(12)	0.099
FULCR	63	0.992	0.008	---	1	(1)	0.016
LC 40	45	0.944	0.056	---	5	(5)	0.106

APPENDIX VI

Allele frequency distribution at the Ck locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Ck^{100}	Ck^{85}	Other	Heterozygotes Observed	(Exp)	Hetero-zygosity
Marine							
ENGLR	30	0.000	1.000	---	0	(0)	0.000
HORSB	37	0.000	1.000	---	0	(0)	0.000
WITLG	33	0.015	0.985	---	1	(1)	0.030
LC 19	57	0.000	1.000	---	0	(0)	0.000
COWIB	27	0.000	1.000	---	0	(0)	0.000
MDERA	--	---	---	---	---		---
SOOKP	33	0.000	1.000	---	0	(0)	0.000
KOKSR	67	0.000	1.000	---	0	(0)	0.000
SLMNC	53	0.075	0.925	---	6	(7)	0.139
GRAPI	41	0.049	0.951	---	4	(4)	0.093
BAMFS	34	0.103	0.897	---	5	(6)	0.185
SARIE	44	0.080	0.920	---	5	(6)	0.147
HAINL	23	0.152	0.848	---	7	(6)	0.258
CONGR	35	0.086	0.914	---	6	(6)	0.157
SANMA	39	0.077	0.923	---	6	(6)	0.142
CHEHW	16	0.031	0.969	---	1	(1)	0.060
Large Lake							
COWIL	36	0.000	1.000	---	0	(0)	0.000
SARIL	57	0.272	0.728	---	21	(23)	0.396
SAKIN	11	0.000	1.000	---	0	(0)	0.000
SPROT	138	0.000	0.996	0.004	1	(1)	0.008
MCRTL	263	0.006	0.992	0.002	4	(4)	0.016
GRCEN	53	0.000	1.000	---	0	(0)	0.000
STELL	18	0.000	1.000	---	0	(0)	0.000
ROBTL	21	0.000	1.000	---	0	(0)	0.000
Small Lake							
CHEML	83	0.000	1.000	---	0	(0)	0.000
FULLL	33	0.000	1.000	---	0	(0)	0.000
MARNL	75	0.000	1.000	---	0	(0)	0.000
LKERR	46	0.000	1.000	---	0	(0)	0.000
HOTEL	114	0.018	0.982	---	4	(4)	0.035
KLEIN	45	0.000	1.000	---	0	(0)	0.000
PAQLK	41	0.000	1.000	---	0	(0)	0.000
TROUT	50	0.270	0.730	---	21	(20)	0.394
GARBY	14	0.000	1.000	---	0	(0)	0.000
BLACK	256	0.000	1.000	---	0	(0)	0.000
PATER	107	0.000	1.000	---	0	(0)	0.000
DEVIL	84	0.000	1.000	---	0	(0)	0.000
SUMNL	66	0.000	1.000	---	0	(0)	0.000
MUDLK	59	0.000	1.000	---	0	(0)	0.000

	LOWRL	47	0.000	1.000	---	0	(0)	0.000
	MCOYL	130	0.000	1.000	---	0	(0)	0.000
	CECIL	103	0.000	1.000	---	0	(0)	0.000
	MORGL	82	0.000	1.000	---	0	(0)	0.000
	ORMND	62	0.000	1.000	---	0	(0)	0.000
	FARWL	139	0.000	1.000	---	0	(0)	0.000
	CEDAR	108	0.000	1.000	---	0	(0)	0.000
	PAXTB	113	0.690	0.310	---	50	(48)	0.428
	PAXTL	44	0.148	0.852	---	9	(11)	0.252
	ENOSB	124	0.940	0.060	---	13	(14)	0.113
	ENOSL	154	0.036	0.964	---	11	(11)	0.069
	GOOSE	67	0.075	0.925	---	10	(9)	0.139
	HECHL	32	0.266	0.734	---	13	(12)	0.390
	CRANL	47	0.617	0.383	---	20	(22)	0.473
Low-lying Stream or Swamp								
	SKOOS	35	0.014	0.986	---	1	(1)	0.028
	SHIPC	27	0.056	0.944	---	3	(3)	0.106
	LC 18	86	0.663	0.337	---	38	(38)	0.447
	GIFFS	13	0.077	0.923	---	2	(2)	0.142
	TEXAS	25	0.140	0.860	---	5	(6)	0.241
	PATBS	49	0.582	0.418	---	21	(24)	0.487
	EBEYI	68	0.000	1.000	---	0	(0)	0.000
	SKAGI	37	0.027	0.973	---	2	(2)	0.053
	MOUSE	36	0.042	0.958	---	3	(3)	0.080
	SMUDL	78	0.538	0.462	---	42	(39)	0.497
	NATHN	41	0.110	0.890	---	9	(8)	0.196
	CLQTZ	47	0.096	0.904	---	9	(8)	0.174
	MCRPD	99	0.066	0.934	---	13	(12)	0.123
	SARIJ	35	0.386	0.614	---	13	(17)	0.474
	TYNEP	288	0.234	0.766	---	101	(103)	0.358
	HOQUB	33	0.864	0.136	---	9	(8)	0.235
	OTTER	115	0.061	0.939	---	14	(13)	0.115
	FARBL	172	0.003	0.997	---	1	(1)	0.006
Isolated Stream or Swamp								
	KEOGH	45	0.000	1.000	---	0	(0)	0.000
	FARSW	75	0.000	1.000	---	0	(0)	0.000
	SPRPD	168	0.000	1.000	---	0	(0)	0.000
	SLZER	55	0.000	1.000	---	0	(0)	0.000
	DRYRN	50	0.000	1.000	---	0	(0)	0.000
Mixed								
	FRASR	106	0.009	0.991	---	2	(2)	0.018
	LARDC	124	0.347	0.653	---	56	(56)	0.453
	FULCR	63	0.063	0.937	---	6	(7)	0.118
	LC 40	45	0.022	0.978	---	2	(2)	0.043

APPENDIX VII

Allele frequency distribution at the Pgm locus. Sample sizes are the number of fish (one-half the number of genes) examined. Expected numbers of heterozygotes (in brackets) were calculated for Hardy-Weinberg conditions.

Population	Sample Size	Allele Frequencies Pgm^{103}	Pgm^{100}	Pgm^{93}	Pgm^{90}	Pgm^{80}	Hetero- zygotes		Hetero- zygosity
Marine									
ENGLR	30	0.000	0.767	0.000	0.200	0.033	11	(11)	0.371
HORSB	37	0.000	0.878	0.000	0.108	0.014	9	(8)	0.217
WITLG	39	0.000	0.744	0.000	0.244	0.013	20	(15)	0.387
LC 19	57	0.009	0.772	0.000	0.219	0.000	22	(20)	0.356
COWIB	27	0.000	0.667	0.000	0.333	0.000	14	(12)	0.444
MDERA	22	0.023	0.750	0.000	0.227	0.000	9	(8)	0.385
SOOKP	32	0.016	0.813	0.000	0.172	0.000	12	(10)	0.309
KOKSR	68	0.022	0.816	0.000	0.154	0.007	19	(21)	0.310
SLMNC	53	0.198	0.575	0.000	0.208	0.019	27	(31)	0.587
GRAPI	41	0.085	0.732	0.000	0.183	0.000	13	(17)	0.423
BAMFS	34	0.029	0.779	0.000	0.176	0.000	12	(12)	0.361
SARIE	44	0.068	0.784	0.000	0.136	0.011	14	(16)	0.362
HAINL	23	0.130	0.804	0.000	0.065	0.000	7	(8)	0.332
CONGR	40	0.075	0.788	0.000	0.125	0.000	16	(14)	0.358
SANMA	46	0.087	0.761	0.000	0.152	0.000	18	(18)	0.390
CHEHW	16	0.000	0.719	0.000	0.219	0.031	6	(7)	0.433
Large Lake									
COWIL	37	0.000	0.689	0.000	0.284	0.027	13	(16)	0.444
SARIL	57	0.158	0.719	0.000	0.123	0.000	25	(25)	0.443
SAKIN	11	0.000	0.955	0.000	0.045	0.000	1	(1)	0.086
SPROT	138	0.051	0.620	0.000	0.301	0.029	71	(72)	0.522
MCRTL	263	0.245	0.618	0.023	0.110	0.004	125	(143)	*0.545
GRCEN	51	0.373	0.529	0.010	0.078	0.010	26	(29)	0.575
STELL	18	0.028	0.694	0.000	0.278	0.000	7	(8)	0.440
ROBTL	21	0.000	0.976	0.000	0.024	0.000	1	(1)	0.047
Small Lake									
CHEML	83	0.831	0.000	0.000	0.169	0.000	26	(23)	0.281
FULLL	33	0.121	0.394	0.000	0.485	0.000	20	(20)	0.595
MARNL	75	0.833	0.020	0.000	0.147	0.000	25	(21)	0.284
LKERR	46	0.087	0.793	0.000	0.120	0.000	15	(16)	0.349
HOTEL	114	0.395	0.048	0.000	0.557	0.000	59	(61)	0.531
KLEIN	45	0.300	0.489	0.000	0.133	0.078	32	(29)	0.647
PAQLK	41	0.000	0.622	0.000	0.378	0.000	17	(19)	0.470
TROUT	50	0.000	0.630	0.000	0.370	0.000	25	(23)	0.466
GARBY	14	0.464	0.321	0.000	0.214	0.000	12	(9)	0.636
BLACK	255	0.143	0.288	0.535	0.033	0.000	139	(155)	0.609
PATER	107	0.397	0.383	0.220	0.000	0.000	72	(69)	0.647
DEVIL	85	0.000	1.000	0.000	0.000	0.000	0	(0)	0.000
SUMNL	66	0.455	0.288	0.053	0.205	0.000	41	(44)	0.665
MUDLK	57	0.272	0.728	0.000	0.000	0.000	23	(23)	0.396

LOWRL	47	0.000	0.986	0.000	0.032	0.000	3	(3)	0.027
MCOYL	130	0.000	0.946	0.000	0.050	0.000	14	(13)	0.103
CECIL	103	0.000	0.704	0.000	0.117	0.180	46	(47)	0.458
MORGL	80	0.225	0.775	0.000	0.000	0.000	30	(28)	0.349
ORMND	62	0.000	0.855	0.145	0.000	0.000	16	(15)	0.248
FARWL	138	0.094	0.254	0.525	0.127	0.000	83	(88)	**0.635
CEDAR	108	0.148	0.593	0.106	0.153	0.000	67	(64)	0.592
PAXTB	93	0.935	0.065	0.000	0.000	0.000	12	(11)	0.122
PAXTL	44	0.239	0.716	0.000	0.045	0.000	17	(19)	0.428
ENOSB	62	0.403	0.573	0.000	0.024	0.000	30	(32)	0.509
ENOSL	154	0.010	0.558	0.000	0.432	0.000	77	(77)	0.502
GOOSE	65	0.346	0.000	0.000	0.654	0.000	25	(29)	0.453
HECHL	37	0.068	0.378	0.000	0.486	0.068	22	(23)	0.612
CRANL	47	0.000	0.628	0.000	0.181	0.000	31	(25)	0.536
Low-lying Stream or Swamp									
SKOOS	34	0.000	0.897	0.000	0.103	0.000	5	(6)	0.185
SHIPC	27	0.000	0.796	0.000	0.185	0.019	7	(9)	0.332
LC 18	87	0.552	0.448	0.000	0.000	0.000	40	(43)	0.495
GIFFS	--	---	---	---	---	---	---	---	---
TEXAS	25	0.180	0.720	0.000	0.100	0.000	10	(11)	0.439
PATBS	49	0.418	0.214	0.000	0.367	0.000	31	(31)	0.645
EBEYI	68	0.074	0.618	0.000	0.301	0.000	33	(35)	0.522
SKAGI	37	0.216	0.581	0.000	0.176	0.027	20	(22)	0.584
MOUSE	36	0.125	0.639	0.000	0.222	0.000	22	(19)	0.527
SMUDL	78	0.654	0.346	0.000	0.000	0.000	30	(35)	0.453
NATHN	41	0.378	0.427	0.024	0.171	0.000	27	(26)	0.645
CLQTZ	47	0.149	0.734	0.000	0.117	0.000	20	(20)	0.425
MCRPD	100	0.260	0.605	0.025	0.110	0.000	58	(55)	0.554
SARIJ	35	0.143	0.800	0.000	0.057	0.000	9	(12)	0.336
TYNEP	323	0.054	0.553	0.000	0.393	0.000	166	(173)	0.537
HOQUB	33	0.000	0.833	0.000	0.167	0.000	9	(9)	0.278
OTTER	117	0.150	0.812	0.000	0.038	0.000	36	(37)	0.317
FARBL	172	0.058	0.230	0.581	0.131	0.000	97	(101)	0.589
Isolated Stream or Swamp									
KEOGH	45	0.433	0.322	0.000	0.244	0.000	27	(29)	0.649
FARSW	71	0.000	0.767	0.227	0.007	0.000	27	(26)	0.360
SPRPD	168	0.003	0.988	0.000	0.009	0.000	4	(4)	0.024
SLZER	61	0.000	0.295	0.000	0.697	0.000	26	(26)	0.427
DRYRN	50	0.000	1.000	0.000	0.000	0.000	0	(0)	0.000
Mixed									
FRASR	105	0.143	0.714	0.010	0.133	0.000	44	(47)	0.452
LARDC	112	0.362	0.554	0.000	0.071	0.009	59	(62)	0.557
FULCR	63	0.016	0.516	0.000	0.468	0.000	28	(32)	0.514
LC 40	42	0.071	0.702	0.000	0.226	0.000	17	(19)	0.451

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* $P < 0.05$

** $P < 0.01$

APPENDIX VIII

Expected mean squares and components of variance for nested analyses of variance performed on transformed gene frequencies of Bear and Somass river system populations. Totals and percentages calculated for components of variance do not include negative values.

Source of Variability	Expected Mean Square
Systems	$S^2(err) + 1.742S^2(loc) + 6.543S^2(hab) + 14.733S^2(sys)$
Habitats	$S^2(err) + 1.727S^2(loc) + 4.181S^2(hab)$
Locations	$S^2(err) + 1.783S^2(loc)$
Error	$S^2(err)$

Components of Variance

	Mdh-1	Mdh-3	Pgi-2	Pgm^{100}	Pgm^{93}
s^2 (err)	.002 (8%)	.003 (2%)	.002 (20%)	.007 (6%)	.003 (3%)
s^2 (loc)	.010 (40%)	.104 (53%)	.006 (60%)	.104 (84%)	.070 (67%)
s^2 (hab)	.013 (52%)	.022 (11%)	.002 (20%)	-.006	-.001
s^2 (sys)	-.006	.067 (34%)	-.002	.013 (10%)	.032 (30%)
Total	.025	.196	.010	.124	.105