ADAPTIVE DIVERGENCE AND THE EVOLUTION OF TROPHIC DIVERSITY IN THE THREESPINE STICKLEBACK

by

PATRICK A. LAVIN

B.Sc., University Of British Columbia 1982

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in
THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA
October 1985

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Department of Zoology

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date Oct 6, 1985

ABSTRACT

Five populations of the threespine stickleback, Gasterosteous aculeatus, from the upper Cowichan River system (Vancouver Island, British Columbia) were surveyed to assess interpopulation levels of variability in trophic morphology. Phenotypic divergence is assumed to be a post-glacial event. Nine characters were scored; eight were related to feeding and the ninth character was lateral plate number. All populations surveyed were the low plate morph; however populations of Gasterosteus in lakes lacking piscivorous fish had significantly fewer lateral plates than populations in lakes with predatory fish species. Three trophic 'morphotypes' were identified, each associated with one of three lake environments. Populations inhabiting benthic dominated environments ('benthic morph') were found to possess reduced gill raker number and reduced gill raker length but increased upper jaw length relative to populations from lentic environments ('limnetic morph'). An intermediate morph may also exist and is characterized by a morphology suitable to either trophic regime.

Analysis of stomach contents showed diet type (benthic or limnetic) to be significantly dependent on morph.

The functional significance of differences in trophic morphology was investigated in three feeding experiments using a representative population from each morphotype. The longer jaw of the benthic and intermediate morphs allowed them to ingest a larger benthic prey than the limnetic. No behavioural component to benthic foraging success between populations was

identified, although increased jaw length shortened the time spent manipulating prey. Both the intermediate and limnetic morphs were better foragers on an experimental limnetic prey than was the benthic. Head length, snout length, gill raker density and gill raker number were strongly correlated with limnetic foraging success.

The quantitative genetics governing the eight trophic characters were investigated using the same three representative populations. Broad sense estimates of character heritabilities ranged from 0.132 to 0.677; all estimates were significant. Character genetic correlations were reasonably strong ($0.3 \leq |rG| \leq 0.9$), while character correlations arising through environment tended to be lower. Cluster analyses of the genetic correlation matrices defined two character suites, the first grouped measures of head shape, the second grouped measures of gill raker structure. The patterns of genetic correlations suggest the three populations are distinct races. Selection gradients for divergence between morphotype indicated that directional selection had operated hardest on head length, snout length, gill raker number, head depth and upper jaw length; hence selection has operated to modify characters related to food size. The benthic-limnetic and intermediate-limnetic morphs were separated by the greatest selection distance while the intermediate-benthic morphs were separated by the shortest selection distance.

These results support the conclusion that directional selection, arising from trophic resource differences between

lakes, has organized interpopulation variability for Gasterosteus within the upper Cowichan drainage. The racial distinction of each population coupled with the functional significance of some components of trophic morphology indicate that at least the benthic and limnetic morphs must be considered 'ecotypes'.

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ACKNOWLEDGMENTS

My initial interest in the stickleback and evolutionary problems was stimulated by Dr. J.D. McPhail. I wish to express my most sincere thanks for his creative insight and patient support in all aspects of this project.

Melanie Madill, Andrew Simons, Eric Taylor and Gary Birch kindly provided of their time for assistance in the field. Melanie Madill deserves special recognition for suffering through boring hours of data recording and key punching.

Drs. Dolph Schluter, Jack Maize and C. Wehrhan led (dragged) me through the mysteries of multivariate statistics. I would particularly like to thank Dolph Schluter for the many hours he devoted to discussing my work, on and off the squash court. Discussions and arguments with Eric Taylor were always enlightening and this work was improved by them.

Comments from Drs. Judy Myers, C. Wehrhan and Nick Giles added clarity to my thinking and this thesis. Bob Carveth's criticisms greatly improved the figures.

Alistair Blachford, Susan Ertis and the staff of the biological sciences data center provided invaluable aid in data analysis.

GENERAL INTRODUCTION

The threespined stickleback (Gasterosteous aculeatus) is a polytypic species (Bell 1976) exhibiting variability in: breeding colours (e.g. McPhail 1969; Moodie 1972); behaviour (e.g. Hay and McPhail 1975; McLean 1980; McPhail and Hay 1983); body morphology (e.g. Hagen and Gilbertson 1972; McPhail 1977; Gross and Anderson 1984); and in biochemistry (Withler and McPhail 1985). Despite the range of characters which show a propensity to vary, the most extensive surveys of variation have concentrated on body armature, particularly with respect to the lateral plates or scutes (e.g. Munzing 1963; Miller and Hubbs 1969; Gross 1977). Recent studies have also examined the loss of skeletal parts including pelvic girdle elements (Giles 1983) and dorsal and pelvic spines (Reimchen 1980b).

Investigations of the three commonly described 'plate morphs' (Hagen and Gilbertson 1972) have generated two hypotheses concerning the origins of freshwater diversity in resident (i.e. nonanadromous) populations of Gasterosteus. The model outlined by Miller and Hubbs (1969) proposes the maintenance of variability by gene flow. The authors suggest that the distribution of plate phenotypes results from continual introgression of freshwater genomes by genetic input from anadromous populations, in this case most of the variation would be neutral. Studies at contact zones however, do not support introgression as hybrids appear to be selectively disfavoured (Hagen 1967). This observation led to the alternate hypothesis proposed by Hagen and McPhail (1970) which describes selection,

as the primary agent organizing freshwater diversity. Later empirical work illustrated the selective advantage of different plate phenotypes (Moodie et al. 1973), while recent studies of coastal Atlantic populations suggest that the partially plated morph (once thought to be a hybrid) may be at a selective advantage in some situations and represent distinct populations (Hagen and Moodie 1982; Wootton 1984).

Although gene flow may not contribute significantly to patterns of interpopulation variability, forces other than selection may still yield detectable variation. Changes in sea-level with temperature minima and maxima during the Pleistocene, afforded anadromous populations saltwater routes into glacial lakes. Freshwater populations are generally thought to be derived from these marine founder stocks (Bell 1976) following the invasion of previously uncolonized habitat; selection subsequently organizes the founder genetic variation. McPhail (1984) has suggested that such founder invasions are responsible for the evolution of the Gasterosteus species pair in Enos Lake, Vancouver Island. These biological species exhibit extreme interspecific divergence in morphology thought to be associated with trophic ecology, and the differences are congruent with diet type. One species, the so called 'benthic' is a bottom browser feeding on macroinvertebrates, while the 'limnetic' feeds almost entirely on planktonic prey. Bentzen and McPhail (1984) have shown differences in jaw morphology, between the two species, to be in part responsible for the dietary distinction. This is one of the few studies involving freshwater populations

of Gasterosteus in which the significance of morphological variability has been clearly defined, but more importantly it indicates a potential mechanism for the evolution of differentiation - adaptive divergence (Bentzen 1982).

Although the species pairs are of great interest they may be evolutionary anomalies and hence provide little generality in describing the origins of racial differences (i.e. variation preserved below the level of biological species). Is there any significance to interpopulation differences or is this variation neutral, arising largely from the effects of history? If adaptive divergence is a common mode of evolution in the stickleback then we must be able to ascribe a significance to the observed variation. This thesis investigates the adaptive divergence in freshwater populations of Gasterosteus.

The study was designed to address three questions relevant to adaptive divergence.

1. How much morphological variation exists within and between resident populations of lake-dwelling Gasterosteus?
2. Does the morphological variation appear to be under genetic control?
3. If selection can be implicated in shifting population morphology, which characters have been selectively modified?

The three chapters which follow focus on each of these questions in turn.

CHAPTER 1

Introduction

The extensive phenotypic variation exhibited by the threespine stickleback Gasterosteous aculeatus, together with the dichotomy between the freshwater and marine forms, has generated two hypotheses to account for the evolution of this diversity. Miller and Hubbs (1969) suggest that much of the phenotypic variation found in freshwater habitats arises from continual introgression from the marine form; whereas, Hagen and McPhail (1970) argue that most freshwater variation is due to local selection. The latter hypothesis is supported by a number of empirical investigations that have identified local adaptations (e. g. Hagen and Gilbertson 1973). Many of these studies focus on body armature, particularly the lateral plate phenotype. This character is easily scored and differences between populations in plate count frequencies are often obvious. Thus, much of the perceived complexity within the Gasterosteous aculeatus complex arises from investigations of plate count frequencies, or the frequencies of different plate morphs. Although this concentration on lateral plates has been productive, it has led to confusion (see Hagen and Moodie 1982 for a discussion of this problem) and, more importantly, it has obscured the extensive morphological variability in other characters, particularly those involved in trophic resource exploitation. This variation is probably adaptive, and if so selection on trophic traits may be a driving force behind

population divergence. Recent studies emphasize the ecological and evolutionary significance of variation in teleost head morphology (e.g. Witte 1984) and in Gasterosteus, differences in head morphology in the Enos Lake species pair appear to be appropriate to their resource use (Bentzen and McPhail 1984).

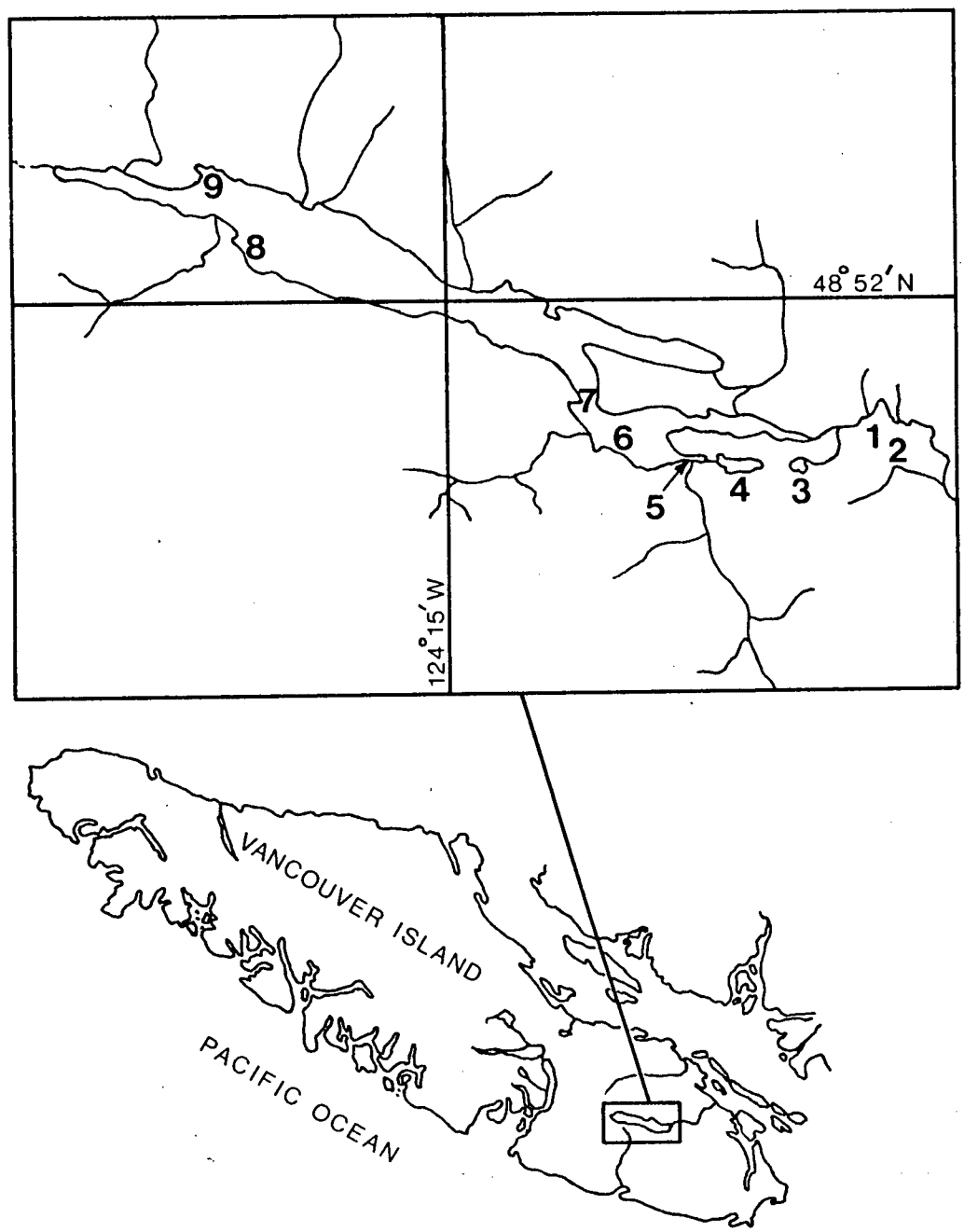
This chapter describes the degree of variation in trophic morphology between populations from five lakes within the Cowichan drainage, Vancouver Island, British Columbia. If interpopulation variation is a response to different selective regimes between lakes, one would predict an association between lake characters and site-specific Gasterosteus morphologies. Thus, I have attempted to identify extant differences in lake characteristics and associate these with divergence in trophic morphology.

Materials and Methods

Cowichan Lake

Cowichan Lake is a large, oligotrophic lake on south-central Vancouver Island, British Columbia. The lake drains through the Cowichan River into the Straight of Georgia (Fig. 1). A recent geologic uplift has caused the river to sink into its own floodplain, confining the river to a narrow, steep channel containing a number of falls (Carl 1953). The anadromous form of Gasterosteus enters the lower Cowichan system but is excluded from my study area by Skutz Falls, a 5.5 metre drop over a 91 metre run. The entire Cowichan Valley was

Figure 1. The Cowichan drainage system and the Strait of Georgia region. (1 = Kwassin Lake, 2 = Grant Lake, 3 = Beaver Lake, 4 = Mesachie Lake, 5 = Bear Lake, 6 = Honeymoon Bay, 7 = Gordon Bay, 8 = Caycuse, 9 = Bay10; 6,7,8, and 9 are all sites within Cowichan Lake.)


9
8
48°52′N
7
6
5
4
3
1
2
124°15′W
VANCOUVER ISLAND
PACIFIC OCEAN

glaciated during the last (Fraser) glaciation and in the initial stages of deglaciation (about 10,000 BP) the study area was covered by a single glacial lake (Alley and Chatwin 1979). This area now contains Cowichan Lake and four smaller lakes (Fig. 1). All of the lakes are interconnected and there are no obvious barriers to stickleback dispersal between the lakes. Thus, gene flow is possible between populations in different lakes. Data on lake morphometry are presented in Table 1.

Table 1. Morphometry data for lakes in the upper Cowichan drainage system.

Lake	Area (Hectares)	Maximum Depth(m)	Elevation (m)
Cowichan	6176.9	45.7	163
Bear	28.0	5.5	163
Beaver	33.0	5.0	181
Grant	2.2	2.5	175
Kwassin	1.4	2.5	175
Mesachie	76.0	10.1	168

The lakes, and sites within the lakes, were grouped by chemical similarity; these groups were then compared to population groupings achieved by morphological analysis, on the sticklebacks collected from each site. Five chemical measures were made at each sampling location: dissolved oxygen, turbidity, conductivity, pH, and alkalinity. Dissolved oxygen was measured in the field, and the four remaining measures were made on water samples returned to the Environmental Engineering Laboratory (U. B. C.). Using Euclidean distance as the similarity criterion the five variables were then entered into a

cluster analysis. Each variable was given equal weight in computing the distance matrix. Euclidean distance is affected by changes in variable scaling; consequently all data were standardized by dividing the ith site datum of the kth variable by the standard deviation of the kth variable (Everitt 1974). Ward's method (Everitt 1974) was used to generate a dendrogram of lake sites (Fig. 2). Eilers et al. (1983) employed a similar classification analysis using three variables to group separate lakes relative to their susceptibility to acidification.

Sticklebacks were collected using pole-seines and minnowtraps from the nine locations during May 1983. All fish were preserved in 10% buffered formalin for one week, washed and then stained in a solution of alizarin red and KOH. Final preservation was in 37.5% isopropyl alcohol. Nine morphological measures were made on each individual ($21 \leq N \leq 40$) with dial calipers ($\pm$ 0.05mm) and where necessary an ocular micrometer. These measures include: standard length (STDLEN), head length (HEAL), snout length (SNOL), eye diameter (EYED), upper jaw length (UPJL), gill raker number (GRN) , gill raker length (GRL), head depth (HEAD), inner orbital width (INOW), and plate number (PLN). Except for plate number all measurments follow Hubbs and Lagler (1958). Plates were scored according to Hagen and Gilbertson (1972). All of these variables, except plates, are associated with trophic exploitation (Kliewer 1970; Fryer and Iles 1972; Northmore et al. 1978; Hyatt 1979; Wright et al. 1983).

Statistical Methods

In animals with indeterminate growth, growth related differences in body size frequently account for the majority of both inter and intrapopulation variability (Thorpe 1976). To remove such size effects each variable was adjusted to a standard length of 40mm. This adjustment uses the linear regression of the log of each variable on the log of standard length (Steele and Torrie 1980). The basic form of the regression is,

$$Yijk = Yjk - \beta jk(Lik - 40)$$

where Yijk is the ith adjusted case of the jth variable in the kth population, Yjk is the sample mean of the jth variable, (Lik - 40) is the standard length of the ith individual minus the grand mean, and βjk is the coefficient of allometry for the jth variable on standard length within each population. Although other authors have adjusted their data sets to a standard length of 50mm (Hagen and Gilbertson 1973; McPhail 1984), some of the populations contained many small individuals (< 35mm) and thus I reduced the adjusted length to 40mm. If the relative growth curves of two populations are similar, but curvilinear; individuals sampled earlier in development yield a steeper function than larger individuals whose growth rate has slowed. As a result, adjusting a sample consisting of many small individuals to a standard length beyond the sample mean, may exaggerate morphological differences between populations. Site specific regression coefficients were used to adjust each character (Thorpe 1976).

Using Sheffe's test, multiple comparisons of sample means were made for each variable. Many of these univariate contrasts were significant ($p < 0.05$), while others suggested certain sites might be grouped by morphological similarity. This possibility was investigated by clustering morphometric data. The methods of this analysis are the same as those used for clustering the lake chemistry data. An element by element correlation of the two Euclidean distance matrices, was used to test for congruence of the two dendrograms.

Patterns of morphological variation were summarized by principal components derived from the character correlation matrix (Pimentel 1979). All characters, except plate number, were entered into the analysis. The contribution of each variable to each component was evaluated by component correlations (Pimentel 1979). To define the relative contributions of intra and interlocality variances to morphological differentiation, an ANOVA was performed on the component scores from the first three components. The integrity of the inferred groupings (see results) was investigated by nesting the populations within the groups suggested by this ANOVA. The sampling program within the Cowichan drainage was not a survey of putative microhabitats; therefore nesting the populations within groups does not violate the assumption of random assignment within a subordinate level (Sokal and Rohlf 1981). Unless otherwise noted, all statistical procedures were performed using MIDAS (Fox and Guire 1976).

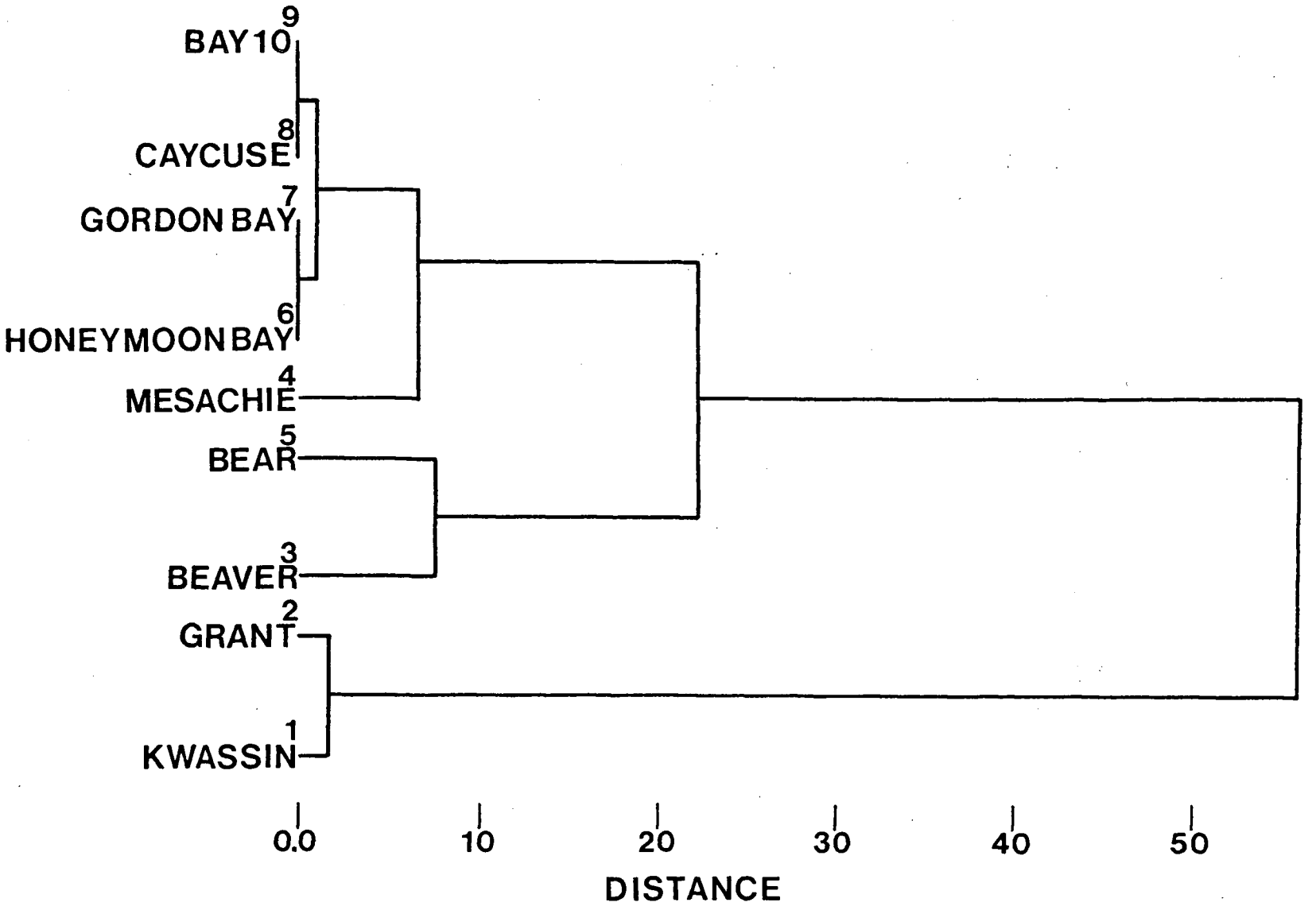
Results

The results of the cluster analysis of site chemistry are summarized in Figure 2. The four Cowichan Lake sites form a distinct group as do Grant and Kwassin lakes. One should note that Bear Lake is more closely related to Beaver Lake even though the former is a small bay off the main body of Cowichan Lake and so might have been expected to group with the Cowichan sites. These results indicate the existence of two lake types defined by chemistry, with the Bear-Beaver pair possibly forming a third type.

For each character and population the coefficients of variation on the unadjusted data are plotted in Figure 3. Such 'variability profiles' (Yablokov 1974) provide two important insights into the nature of evolutionary responses: (a) an indication of character correlation, and (b) the mechanism by which a particular species interacts with selective constraints. Concordance of peaks and troughs, but differences in peak amplitudes, suggests that the species is responding to local selection with a common genetic architecture. This is in contrast to genome reorganization as a response to local selection (Sokal 1978). These concepts are treated in detail below.

For each variable and each population the adjusted means and standard deviations are reported in Table 2. There were no significant differences ($p > 0.05$) between subsamples within Cowichan Lake; however, all Cowichan subsamples were significantly different ($p < 0.05$) from all other lakes in at

Figure 2. Dendrogram summary of lake groupings based on lake chemistry.


9
BAY 10
8
CAYCUSE
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GORDON BAY
6
HONEYMOON BAY
4
MESACHIE
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BEAR
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BEAVER
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GRANT
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KWASSIN
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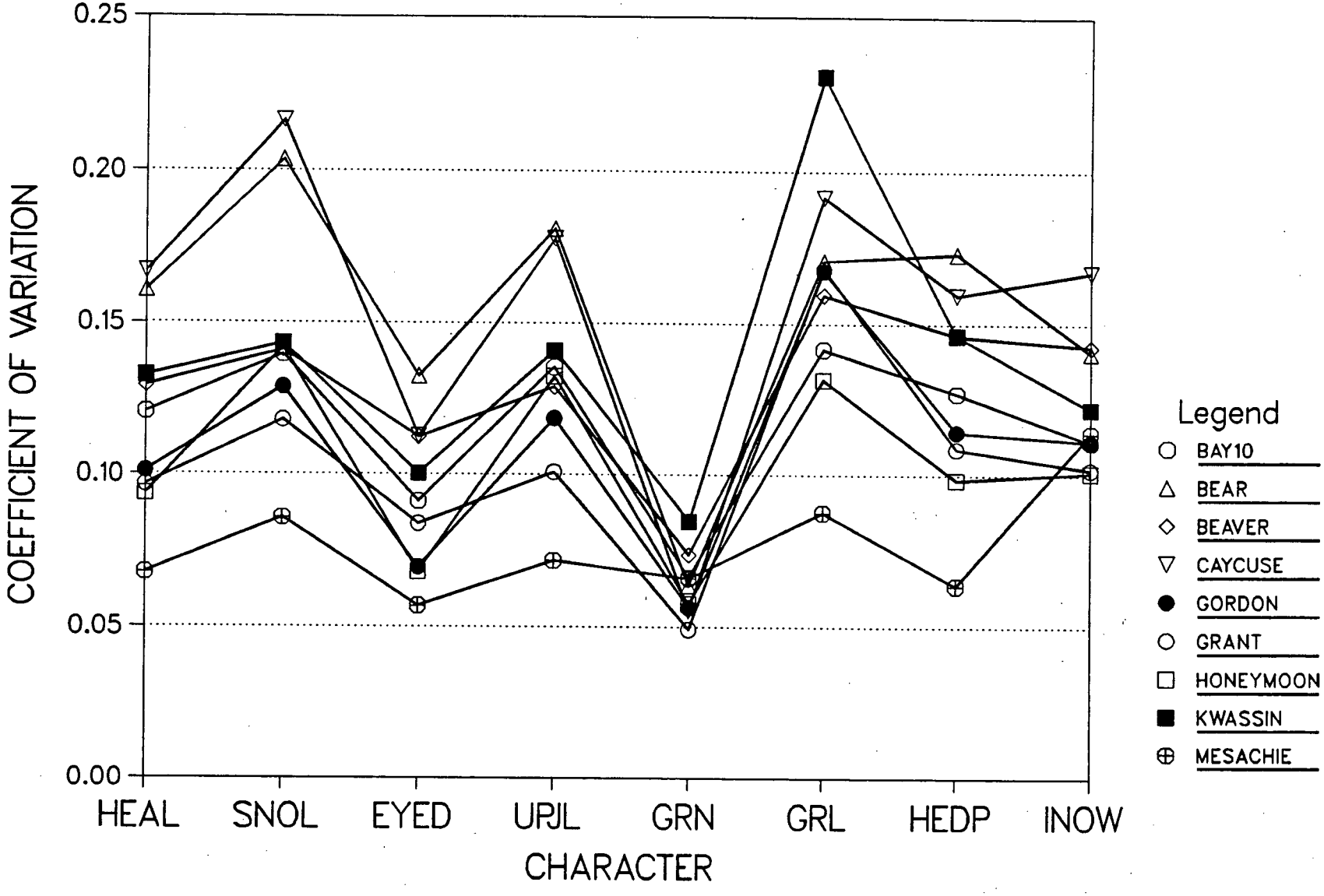
DISTANCE

Figure 3. Variability profiles for the nine sampling sites in the Cowichan drainage.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COEFFICIENT OF VARIATION
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HEDP
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Legend
BAY10
BEAR
BEAVER
CAYCUSE
GORDON
GRANT
HONEYMOON
KWASSIN
MESACHIE

least one variable. Mean plate number is also presented in Table 2 for each population. All populations, including sites within Cowichan Lake, are clearly the "low plate morph" (Hagen and Gilbertson 1972), although there are significant differences between populations in mean plate number. For both Beaver and Bear lakes mean plate numbers do not differ from any of the Cowichan Lake sites or from Mesachie Lake; however the plate means for both Grant and Kwassin lakes are lower than all other samples ($p < 0.0001$). Neither Grant nor Kwassin Lakes contain any species of piscivorous fish although stickleback populations in both lakes are subject to avian predation. All of the other lakes contain a variety of fish species known to prey on Gasterosteus.

These univariate comparisons suggested that populations within the smaller lakes (Bear, Beaver, Grant and Kwassin) were morphologically distinct from both the Cowichan Lake sites and the Mesachie Lake sample. The dendrogram derived from the character data support this conclusion (Figure 4). The Cowichan Lake sites and Mesachie Lake form one cluster while the smaller lakes form a second cluster. Interestingly, the analysis preserves the grouping of Bear and Beaver lakes produced by the clustering of site data, although in this instance the pair cluster more closely with the Kwassin-Grant group. The distance matrices were reasonably strongly correlated ($r = 0.653$).

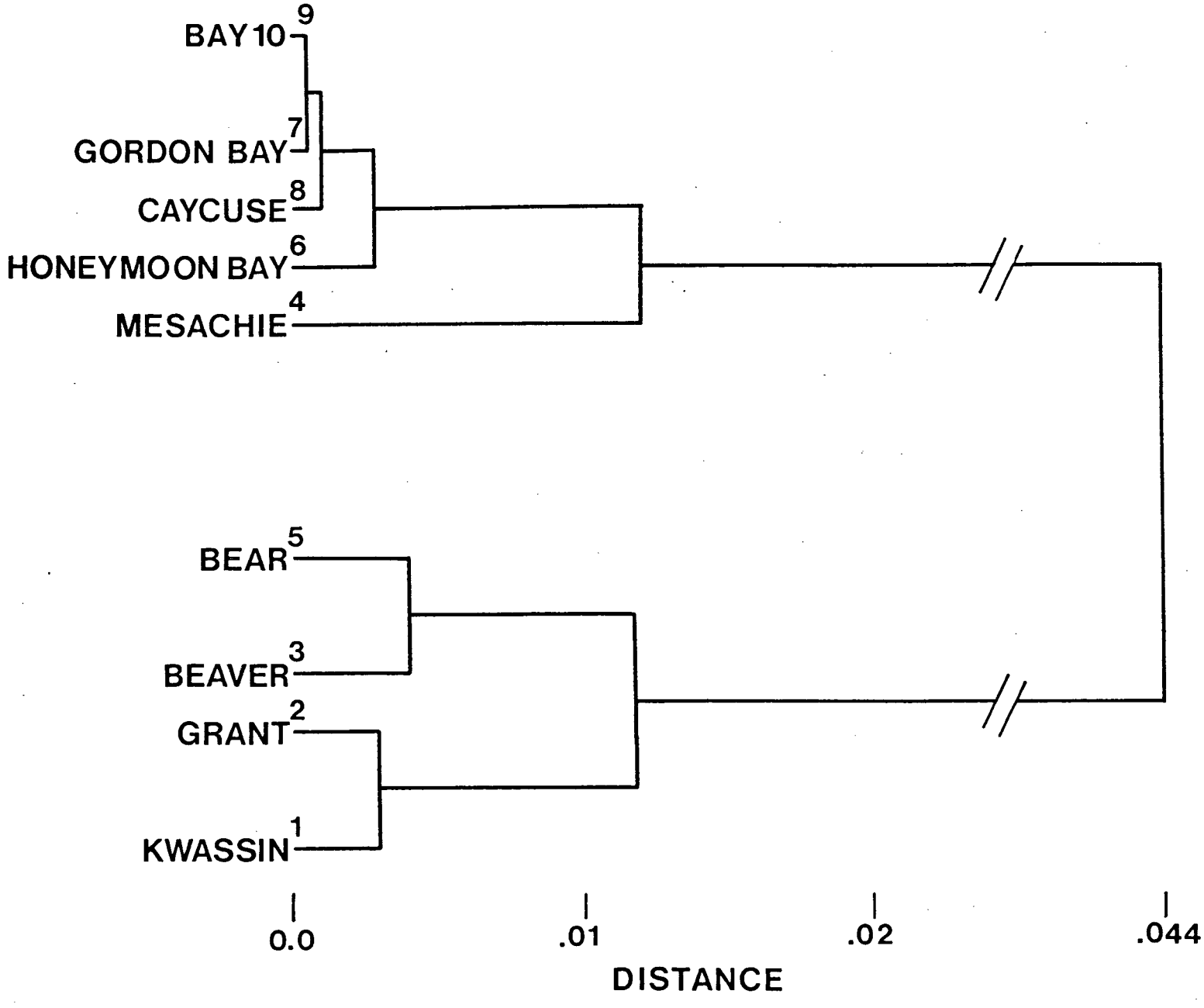
Clearly, within the upper Cowichan system there exist at least two morphologically distinguishable groups. Is this grouping site-specific or are the populations simply components

Table 2. Means and standard deviations on adjusted data for each variable and population. All data adjusted to 40mm standard length. Standard deviations are given in brackets.

Table 2. Population means and standard deviations for all adjusted variables.

Population	N	HEAL	SNOL	EYED	UPJL	GRN	GRL	HEAD	INOW
Bay10	21	11.98	3.73	3.94	2.89	20.10	0.84	5.55	2.45
		(.51)	(.15)	(.16)	(.25)	(1.33)	(.09)	(.25)	(.13)
Bear	30	12.47	3.67	3.81	3.04	17.86	0.80	5.55	2.38
		(1.27)	(.24)	(.21)	(.20)	(1.13)	(.11)	(.32)	(.16)
Beaver	23	11.93	3.71	3.73	2.94	18.60	0.73	5.47	2.16
		(.50)	(.20)	(.16)	(.19)	(1.37)	(.08)	(.27)	(.14)
Caycuse	30	11.68	3.62	3.95	2.95	19.70	0.85	5.50	2.37
		(.42)	(.25)	(.16)	(.20)	(1.08)	(.10)	(.26)	(.15)
Gordon	29	11.84	3.75	3.97	2.83	20.17	0.84	5.36	2.37
		(.45)	(.21)	(.17)	(.18)	(1.13)	(.10)	(.27)	(.14)
Grant	30	12.60	3.62	4.06	3.39	17.46	0.69	5.70	2.21
		(.60)	(.32)	(.17)	(.22)	(.86)	(.11)	(.36)	(.16)
Honeymoon	21	11.25	3.50	3.88	2.80	19.35	0.83	5.31	2.35
		(.56)	(.33)	(.19)	(.28)	(1.11)	(.08)	(.31)	(.17)
Kwassin	30	12.24	3.99	3.95	3.33	17.30	0.71	5.79	2.35
		(.67)	(.33)	(.20)	(.27)	(1.46)	(.15)	(.34)	(.19)
Mesachie	30	12.12	3.73	3.85	3.10	19.06	0.97	5.75	2.33
		(.45)	(.15)	(.16)	(.11)	(1.25)	(.07)	(.21)	(.22)

Figure 4. Dendrogram summary of lake groupings based on population morphology.


BAY 10
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CAYCUSE
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HONEYMOON BAY
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MESACHIE
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BEAR
5
BEAVER
3
GRANT
2
KWASSIN
1
0.0
.01
.02
.044
DISTANCE

of a linear array that has arisen through stochastic events? Linear clinal variation could result from gene-flow between subpopulations that possess different allele frequencies as a result of genetic drift or founder effect (Endler 1977). The interpopulation phenotypic correlation matrix contained only eight (of a possible 28) significant ($p < 0.05$) correlations. This suggests that certain phenotypic characters might be responding to similar influences (genetic, environmental, or both) across habitats and therefore acting as a character suite. In contrast to the interpopulation character correlations, all the intrapopulation correlation matrices contained at least eight, and as many as twenty, significant correlations. This pattern strongly suggests that the population phenomes are responding to some site-specific influence. Further, the pattern of reduced interpopulation character covariance indicates that population divergence in this system may be the result of directional selection acting on a limited suite of correlated characters. As a result, the next issue addressed was the identification of phenotypic character suites and an investigation of their relative contribution to the observed population differentiation.

Initially, principal components were extracted from the individual populations and variable loadings compared for the first component. The first principal component accounted for 40-70% of the total variance across the nine populations with HEAL, SNOL, UPJL and HEAD consistantly having the highest coefficients. As a result, the populations were pooled and

components extracted from the total pooled correlation matrix. Table 3 summarizes the results of this principal components analysis.

Table 3. PCA from adjusted data (all variables except plate number).

	Principal Components		
Variable	Axis 1	Axis 2	Axis 3
HEAL	0.41699	-0.18473	0.10923
SNOL	0.45125	0.14857	0.19395
EYED	0.31298	0.00425	-0.83628
UPJL	0.43590	-0.29276	0.05008
GRN	-0.10718	0.60835	-0.27465
GRL	0.16016	0.55495	0.39478
HEAD	0.45600	-0.03063	0.09243
INOW	0.29907	0.42317	-0.09353
Eigenvalue	3.5524	1.7035	0.7939
%variance	44.40	21.50	9.92

The first three components account for 75.62% of the total variance. As a result of the initial adjustment of the data set to a grand mean of 40mm, all components must be representations of shape differences. Table 4 presents the correlation coefficients between the ith original variable and the jth component. Head depth, snout length, upper jaw length and head length are all highly correlated with the first component which may be thought of as a summary variable describing head shape. Character correlations tend to decrease on the following two components. The component correlation is often considered to be the ith variable's response to the jth stimulus (Morrison 1967) ; consequently as the proportion of variance accounted for

Table 4. Correlation coefficients between each character and principal component.

Variable	Eigenvector Axis 1	Axis2	Axis3
HEAL	0.7859	-0.2411	0.0973
SNOL	0.8505	0.1939	0.1728
EYED	0.5899	0.0056	-0.7452
UPJL	0.8215	-0.3821	0.0446
GRN	-0.2020	0.7940	-0.2447
GRL	0.3018	0.7243	0.3518
HEAD	0.8594	-0.0474	0.0824
INOW	0.5636	0.5523	-0.0833

decreases (i. e. the effect of the major stimuli are removed) correlations of any given variable are likely to decline. There remain however, three relatively high correlations on the next two axes: gill raker number and gill raker length on the second axis and, eye diameter on the third axis. These responses should not be dismissed as they may be the features producing the group differentiation outlined below.

The distribution of variance summarized by the first three components was examined by ANOVA. PCI accounts for 44.4% of the total variation; 21.0% of this proportion is a result of variation among populations and the remainder is due to within population variance. PCII accounts for 21.5% of the total variation: 63.9% results from differences among populations and suggests that gill raker number and length, may be important aspects of population divergence. Upper jaw length contrasts with gillraker number and length on this component (Table 3). Populations from the small shallow lakes (Bear, Beaver and Grant) tend to have longer jaws but reduced gillraker number and

length (Table 2) compared to populations from the larger, deeper lakes (Cowichan and Mesachie).

Finally, PCIII accounts for 9.9% of the total variation; 34.7% of this proportion results from differences among populations. In summary, 26.5% of the variance summarized by the first three components, arises from differences among populations.

The means and standard deviations of the component scores are plotted in Figure 5. Although the intrapopulation variation reduces group discrimination on the first axis, the second axis appears to yield the separation of at least two groups. The intermediate populations may, or may not, represent a third grouping. To investigate the integrity of these inferred groups, a nested ANOVA was performed on component scores, nesting populations within lake groupings (UBC:GENLIN). The design of this analysis is given in Table 5. Since Bartlett's test indicated that the variance among the lake groupings did not violate the assumption of homoscedasticity; Tukey's multiple range test was used to identify differences among sites. Tukey's HSD identified two homogeneous subsets among PCI scores - [2,1] and [3], ($p < .05$). Significant variation was also found among sites nested within groups. This result was expected as the initial single classification ANOVA had already demonstrated significant within group variance on PCI. Nested ANOVA on the scores from PCII yielded significant differences among groups ($p = 0.000$) with much reduced within site variation ($0.0 < p < 0.05$). In this instance Tukey's test indicated

Figure 5. Bivariate mean component scores, for each population, plotted on the first two principal components. Glyphs indicate mean position for each population; black bars indicate one standard deviation on either side of the mean.

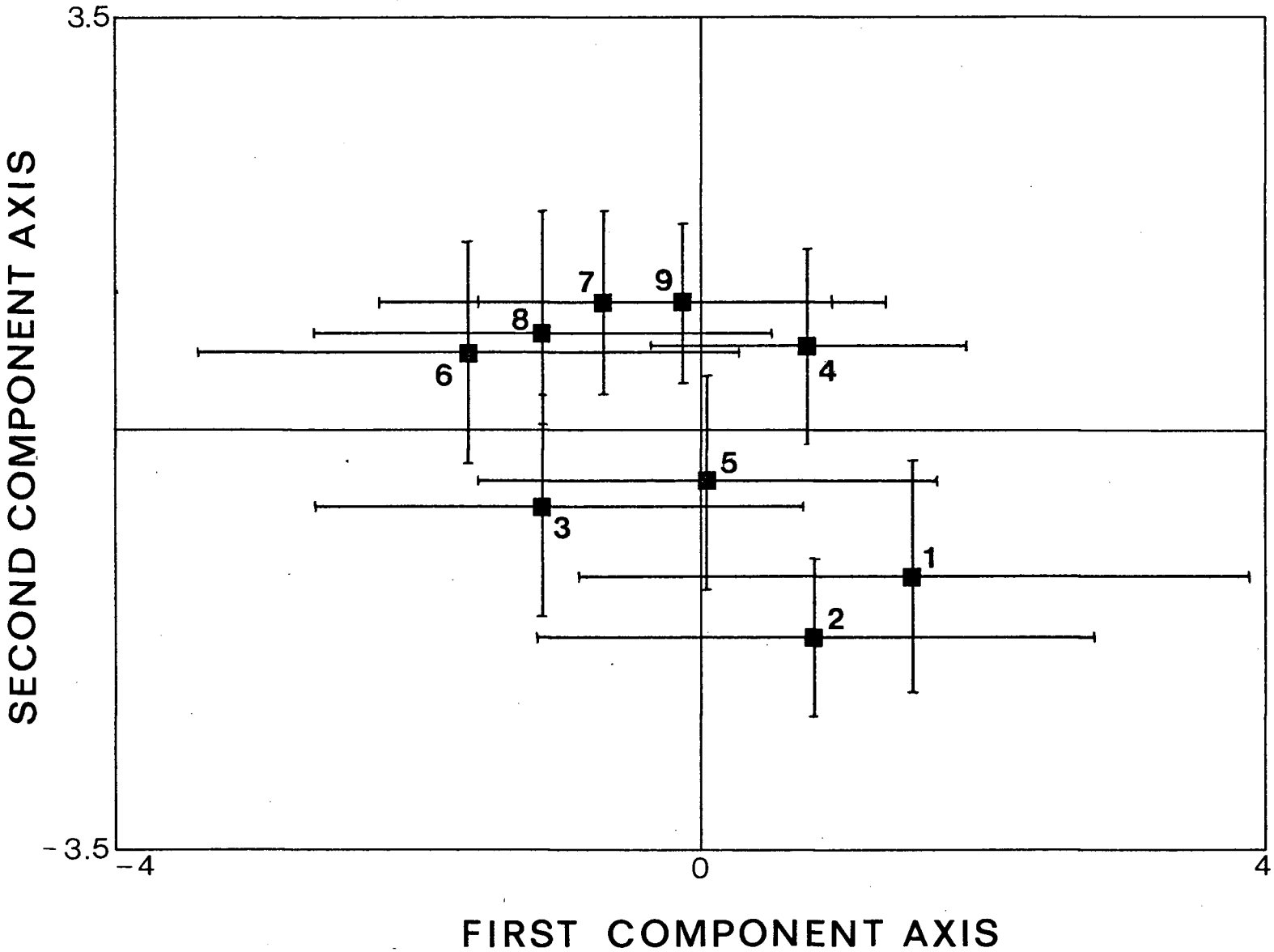

3.5
-3.5
-4
0
4
SECOND COMPONENT AXIS
FIRST COMPONENT AXIS
7
9
8
6
4
5
3
1
2

Table 5. Design of the nested ANOVA on component scores from the first three eigenvectors.

Lake Group		
1	2	3
Caycuse	Beaver	Grant
Bay10	Bear	Kwassin
Gordon Bay		
Honeymoon Bay		
Mesachie		

three homogeneous subsets - [3], [2], and [1]. Scores from PCIII produced significant differences both between and within lake groups ($p = 0.000$) and two subsets were indicated - [3,1] and [2].

Discussion

Here I have attempted to address two issues: (a) the multivariate response of Gasterosteus populations to some organizing forces, and (b) the characterization of these forces as local selective effects. There is clearly a multivariate, site-specific, morphological differentiation within the Cowichan drainage system and the distribution of these morphologies is congruent with lake differences. Given this result, to what subset of lake differences are the phenomes responding? All the variables scored, except lateral plates, are associated with teleost trophic ecology. Consequently the 'latent factor variables' (Morrison 1967), described by the three axes must be multivariate summaries of trophic morphology. Hence, the observed population divergence indicated by these summaries is the phenotypic response of each population to some inherent

(site-specific) stimulus. Gill raker architecture has been implicated in planktivory in a variety of teleosts (Kliewer 1970; Magnuson and Heitz 1971; Wright, et al. 1983); populations inhabiting pelagic regions are found to possess long and numerous rakers, while populations in habitats dominated by benthic production are characterized by shorter and fewer rakers. This pattern has been noted for Gasterosteus both in North America (Hagen and Gilbertson 1972) and Europe (Gross and Anderson 1984). In the Cowichan system gill raker architecture and head morphology are associated with site type and I suggest that the observed differences, although small compared to intrapopulation variance, are a response to trophic differences between sites.

Both Cowichan and Mesachie lakes are dominated by lentic environments with comparatively little littoral development. Gasterosteus in these lakes are open water pelagic foragers, rarely found close to shore except during the breeding season. The diet of these animals is dominated by copepods and cladocerans (Carl 1953). In contrast Grant and Kwassin lakes are very shallow with no appreciable lentic regions, and these populations feed primarily on macroinvertebrates (Chapter 3). Feeding studies have shown that the jaw morphology of individuals from Grant Lake allows them to ingest significantly larger prey than those from Cowichan Lake (Chapter 3). The differences in trophic morphology between populations therefore appear to be ecotypic. At this point however, I will forego the use of the term 'ecotype' and instead define three morphotypes:

a limnetic, a benthic and an intermediate. The limnetic morph includes all samples from Cowichan Lake in addition to the Mesachie Lake population; the benthic morph includes both Grant and Kwassin lakes; while the intermediate morph describes populations from Bear and Beaver lakes.

The patterns of character covariance summarized by the PCA are particularly interesting as they give a statistical measure of the degree to which the phenotype is integrated (Sokal 1978). Gill raker number and gill raker length appear to form a character suite independent of head shape described by the first component. However, to extend evolutionary arguments from patterns of phenotypic covariance, it is necessary to have some indication that the pattern has a genetic basis. The variability profiles (Fig 3.) in this study suggest that there is a genetic component to each of the characters scored. It is unlikely that the observed concordance of profiles between populations would exist without a genetic component, as this would require similar sets of environmental constraints acting simultaneously on all populations. Sokal (1978) considers it unlikely that one could find functionally independent characters under simultaneous selection across populations, due to the 'cost of selection' argument. Populations are thought to be unable to suffer the genetic load associated with simultaneous selection on a host of genetically independent characters (Futuyma 1979). If the genotype in a given population is integrated by linkage disequilbrium and/or pleiotropy, the number of selective deaths per generation decreases relative to

a population containing genotypes controlled by large numbers of independent genes. Pleiotropy and linkage lead to character correlation (Falconer 1981), hence we have an initial indication that these trophic characters are probably at least in part, genetically correlated. Correlations are treated in greater detail in Chapter 2.

Although plate numbers vary between the lakes, plate phenotype is an inadequate descriptor of the interpopulation variation within the Cowichan drainage. Within the system, selection on plates appears to be independent of selection on trophic morphology. This suggests two independently evolved character suites. Unfortunately this result may be biased. No predatory fish occur in either Grant or Kwassin lakes and thus the conclusion that plate phenotype evolved independently of the trophic character suite is dependent on the questionable intermediate morphology of the Beaver and Bear lakes populations. Perhaps plate phenotype is pleiotropically linked to trophic morphology. Within populations both scute and spine phenotypes appear to distribute themselves differentially among sites within populations (Moodie 1972; Larson 1976; Reimchen 1980a). These distributions may indicate selection for specialist phenotypes each adapted to a restricted segment of the total lake habitat (i.e. 'Niche variation hypothesis', Van Valen 1965). This may be the mechanism preserving relatively high intrapopulation variation in Gasterosteus (Reimchen 1980b).

At all sites, with the exception of Grant and Kwassin, lateral plate number shows a strong mode at seven. This

arrangement is associated with the presence of piscivorous fish (Hagen and Gilbertson 1973), and plate phenotype has a modest heritability (Hagen 1973). The low plate numbers in Grant and Kwassin lakes are associated with the absence of predatory fish species; however, both lakes contain high densities of invertebrate predators (Lethocerus americanus, Dytiscus sp., Aeshna sp. and Libellula sp.) and I have observed invertebrate attacks on Gasterosteus in both lakes. Recently, Reimchen (1980b) has suggested that reduced body armature may be a response to invertebrate predation.

Very little of the less obvious morphological variation has been investigated in Gasterosteus, and evolutionary narratives for this species usually extend from more distinct differences. The biology of Gasterosteus, however, is such that inferences based on plate phenotypes and plate frequencies may confuse the effects of selection, hybridization and history on variation. The concordance of morphology and habitat described above, suggests that interpopulation differentiation in this system is a response to different selective regimes. Two predictions originate from this hypothesis.

1. The characters measured must have a genetic component if they are to evolve in response to selection.
2. If a given trophic character(s) has been organized by selection between populations, one should be able to demonstrate the functional significance of that character by contrasting its performance in different environments.

Both of these predictions are tested in the following

chapters.

CHAPTER 2

Introduction

Recent criticisms of the inability of evolutionary studies to clearly define the target features of natural selection (Lewontin 1978; Gould and Lewontin 1979), has led to a reexamination of organismal design. Studies that atomize the phenotype into smaller and smaller subunits may obscure the processes of selection active at the interface of phenotype and environment (Bock 1980; Mayr 1984). Hence, some degree of 'holism' is demanded if one is to properly define processes of morphological evolution. Methodologies for such an approach have only recently been described and are based on the recognition of organisms as integrated functional units which evolve (Gould and Lewontin 1979). Consequently all phenotypic characters (despite the organizational level at which they are defined) necessarily evolve only within the context of the organism (Cheverud 1982).

The implications of phenotypic integration are not newly recognized, Darwin first suspected their existence in 1859,

> "...the whole organism is so tied together...that when slight variations in one part occur, and are accumulated through natural selection, other parts become modified. This is a very important subject, most imperfectly understood." p182.

Morphological integration is thought to arise primarily through the effects of pleiotropy and linkage (Falconer 1981), which yield character correlations. In most studies, workers have

sought to identify patterns of character correlations in polygenic characters, for which pleiotropy may be most important. Working with polygenic characters has two advantages: most evolutionarily important characters are thought to be polygenic (Lewontin 1974); the genetics of polygenetic systems has been extensively treated in the literature, and is based largely on parametric statistics. This statistical background has become the foundation for testing hypotheses concerned with character integration. Indeed, Cheverud (1982) feels strongly that the 'degree of integration' may be measured by the statistical association of the phenotype, and several workers (Leamy 1977; Atchley and Rutledge 1980; Atchley 1981; Cheverud 1981, 1982; Leamy and Atchley 1984) have concentrated efforts on describing these associations.

In this chapter I present the results of a laboratory breeding program which was designed to address three questions:

1. How much genetic variation is present within each morphotype;
2. How are the characters organized to respond to selective constraints; and,
3. How have characters responded to selection during the course of the population's evolution?

Materials and Methods

Establishment and Fostering of Progeny

Logistics prevented raising progeny from all five lakes, I therefore chose to limit the breeding study to three populations; one from each of the proposed morphotypes. The limnetic representative was from the north end of Cowichan Lake (Caycuse), the benthic form was from one of the small bog lakes (Grant), and the intermediate form was taken from the small bay off the main body of Cowichan (Bear Lake).

Mature adults were collected from three sites in June and July (1984) using minnow traps and pole-sienes. Adults were chosen without bias from the fish collected at each site, but some effort was made to choose individuals which represented the observed range of standard lengths in the breeding population. Fertilization of eggs was done on site following the methodology outlined by Bell (1984), but using dechlorinated water transported from the laboratory, rather than lake water. This precaution was taken in order to reduce possible effects of lake water on development, particularly those effects resulting from differences in temperature and/or the possible introduction of fungus. I attempted to make a minimum of 12 crosses from each population; 14 families were obtained from Grant Lake, 12 families from Bear Lake and 12 families from Caycuse. Although laboratory mortality was generally low, fungus killed 2 families from Caycuse leaving only 10. The paucity of breeding adults at this site precluded the replacement of these two families.

Fertilized eggs and parents were returned to the laboratory. Egg batches were incubated in a water bath at 17.5°C until hatch (approximately seven days after fertilization). Newly hatched fry were left in the incubator for three days following hatch, and then moved into the light for swimup. After swimup, individual families were placed in 20 litre aquaria. Illumination was by fluorescent lights mounted eight inches above the tanks. A constant light-dark cycle (16 hours light: 8 hours dark) was maintained for the entire lifetime of the progeny. Fry were fed an infusoria culture for approximately one week, or until the fry could ingest Artemia nauplii. Once the fry attained approximately 20 millimetres standard length, their diet was switched to a mixture of Tubifex, chopped liver, and frozen Artemia. Large families (> 40 individuals) were split into subfamilies by removing fish at random and placing them in other tanks. This subdivision provided the advantage of reducing the effects of common environment on the estimate of heritability (Falconer 1981). All families were maintained until February (1985) and then sacrificed and preserved. Progeny and parents were scored for the same set of characters as those given in Chapter 1. Standard lengths of progeny varied widely, within and between populations, hence all measures were again adjusted to 40 millimetres. All adjusted data were log (base e) transformed before proceeding with any analysis.

Estimation of Character Heritabilities and Correlations

The heritability of any polygenic character is defined as the ratio of additive genetic variance (Va) to the character's phenotypic variance (Vp). Heritability in the 'narrow sense' is given as Va/Vp and may be estimated by a variety of experimental designs (Falconer 1981). Initially I had hoped to determine a narrow sense heritability for each character using midparent-offspring regression (Falconer 1981). Unfortunately the intraclass correlation coefficient (described below) was found to be low for most characters and as a consequence 10-14 families were not enough to obtain a reasonable estimate of Va by regression. Heritability was therefore estimated from ANOVA using the progeny of the single pair matings (Becker 1975). Table 6 outlines the design of the analysis and the expected mean squares.

Table 6 Design of fullsib ANOVA for the estimation of Vg.

Source of Variation	Expected Mean Square
Among Families	$\sigma^2w + No(\sigma^2a)$
Within Families(error: among individuals)	σ^2a

* No is the weighted average family size.

In this design H^2 is estimated from the intraclass correlation coefficient (t) where: $t = S^2 / S^2 + S^2w$, and S^2 estimates σ^2a while S^2w estimates σ^2w. In this instance H^2 is given as : $H^2 \leq 2t$ (Falconer 1981). The inequality results from

the estimate of $\sigma^2 a$. $S^2 a$ estimates 1/2 the additive genetic variance but also 1/4 of the dominance variance (Becker 1975). The measure of heritability is therefore defined as 'broad sense' and given as Vg/Vp, where Vg = 1/2(Va) + 1/4(Vd) + Vi. Vi is a measure of all variance arising from non-additive effects (Hartl 1979). As a consequence of this estimate, H^2(obtained) sets only an upper limit to H^2(population). If the dominance deviations are zero then the estimate approximates the measure of narrow-sense heritability, except for the additional interactions. Nevertheless, the broad sense estimate of H^2 provides the basis for the construction of evolutionary inference.

The estimation of additive genetic covariance between characters proceeds in the same manner as the estimation of genetic variance, but in this instance the sums of cross-products are partitioned rather than the sums of squares. Becker's computational formulae (1975) were used for partitioning the sums of cross-products. Three correlations were estimated for each character pair (x,y), within each population:

1. Genetic: $$\frac{r(G) = 2\mathrm{cov}(a)}{(2\sigma^2 a(x) * 2\sigma^2 a(y))^{.5}},$$

2. Phenotypic: $$\frac{r(P) = \mathrm{cov}(w) + \mathrm{cov}(a)}{[(\sigma^2 w(x) + \sigma^2 a(x)) * (\sigma^2 w(y) + \sigma^2 a(y))]^{.5}},$$

3. Environmental: $$\frac{r(E) = \mathrm{cov}(w) - \mathrm{cov}(a)}{[(\sigma^2 w(x) - \sigma^2 a(x)) * (\sigma^2 w(y) + \sigma^2 a(y))]^{.5}},$$

where cov(a) estimates the covariance between characters among families and cov(w) estimates the covariance between characters within families. The variance terms are the same as those

estimated in the previous ANOVA. Again, as in the estimation of H^2, the numerator in these equations contains dominance and epistatic effects, in addition to additive effects.

Interpreting patterns of single correlations between pairs of characters is hindered by two factors. The first is that single genetic correlations will vary due to differences in gene frequencies, selective regimes and evolutionary history (Atchley et al. 1981). The second difficulty results simply from the inability to define and describe patterns arising from multiple effects (in this case 28 genetic correlations, within each population); hence it is preferable to apply some kind of summary technique (Oxnard 1978).

Boag (1983) has extracted principal components directly from the correlation matrix, however this method is not favoured as genetic correlation matrices are often illsuited for PCA (Leamy 1977 ; Boag 1983). In contrast to PCA, cluster analysis may be used with many kinds of similarity matrices, including genetic correlations, and is less rigid in its assumptions concerning the nature of the input matrix. Cluster analysis was therefore used to summarize patterns of genetic correlations using 'complete linkage' as the clustering algorithm (Everitt 1977). Boag (1983) has used 'average linkage' to cluster correlation data, but both average linkage and single linkage gave results very similar to those of complete linkage using my data. The statistical package 'S' (Becker and Chambers 1984) was used to compute the character linkages.

Reconstructing the Pattern of Trophic Divergence

In the past the process of reconstructing historical patterns of morphological divergence has been complicated by the observation that continuously varying traits tend to covary; consequently, it is difficult to identify those characters which might be considered the targets of natural selection (Arnold 1983). If the targets of selection can not be elucidated, then the processes of morphological evolution necessarily remain obscure (Gould and Lewontin 1979 ; Bock 1980). Recently Russell Lande (1979) has provided a multivariate solution for describing the evolution of correlated characters. He defines a selection gradient for the evolution of mean phenotype as:

$$\beta = G^{-1}[z(0) - z(t)],$$

where G^{-1} is the inverse of the symmetric matrix of character variances and covariances; z(0) and z(t) are vectors of character means for the population at times '0' and 't'. If the structure of the character complex, as described by 'G', is constant over evolution, then the net selection gradient is the sum of $\beta = G^{-1}\Delta z$ over the generations '0' to 't - 1'. This measure of selection is independent of the path taken between z(0) and z(t) and is therefore robust to changes in the rate and direction of evolution (Lande 1979). Given the assumption of the constancy of 'G', we may calculate selection gradients between populations (or species). In this instance vectors of character means for population's 'a' and 'b' are substituted for z(0) and z(t). If the individual vectors z(a) and z(b) are transformed such that:

$z^* = G^{-1}$ then,

$\beta = z^*(a) - z^*(b)$ (Schluter 1984).

The elements βi of the selection gradient are the net forces of natural selection which have acted on each character independent of the correlated responses to selection on the other characters measured. Recently Schluter (1984) has defined the length of the vector β as the Euclidean distance

$$B = [\Sigma(z'i(a) - z'i(b))^2].$$

B then, is the net force of directional selection which would be required to shift mean morphology from z(a) to z(b). If 'G' is known, selection gradients between pairs of populations may be calculated under the assumptions that 'G' has been determined without error, and that it has remained constant through time (Price et al. 1984). Given the preceding assumptions, I have calculated net selection gradients for population transitions from the pooled within populations matrix of genetic variances and covariances (Schluter 1984).

Results

Heritability and Genetic Correlations

The means and standard deviations of both the adjusted progeny values and the wild population values are given in Table 7. For the progeny data, there are four more significant ($p < 0.05$) character contrasts between populations than for the wild data. This suggests that selection and/or environment may be masking some interpopulation differences in gene frequencies.

Table 7. Descriptive statistics for wild collections and laboratory reared progeny.

Table 7. Means and standard deviations of adjusted data for each character and population.*

I. Wild Populations

Character	Population: Bear Lake(a) N = 30	Caycuse(b) N = 30	Grant Lake(c) N = 30
HEAL (ac)b#	2.52(0.092)	2.46(0.036)	2.53(0.053)
SNOL (abc)	1.29(0.065)	1.29(0.068)	1.28(0.090)
EYED abc	1.34(0.058)	1.37(0.042)	1.40(0.043)
UPJL (ab)c	1.11(0.065)	1.07(0.071)	1.22(0.066)
GRL (ab)c	-0.23(0.131)	-0.16(0.126)	-0.37(0.167)
GRN (ac)b	17.86(1.130)	19.17(1.080)	17.46(0.860)
HEAD (ab)c	0.86(0.071)	0.86(0.067)	0.79(0.076)
INOW (ab)c	2.88(0.063)	2.98(0.055)	2.86(0.049)

II. Laboratory Progeny

Character	Population: Bear Lake(a) N = 357	Caycuse(b) N = 179	Grant Lake(c) N = 292
HEAL abc	2.54(0.038)	2.56(0.034)	2.56(0.049)
SNOL abc	1.45(0.061)	1.49(0.067)	1.42(0.062)
EYED (ab)c	1.39(0.053)	1.39(0.063)	1.41(0.052)
UPJL (ab)c	1.13(0.072)	1.13(0.083)	1.17(0.079)
GRL (ac)b	-0.06(0.115)	-0.01(0.120)	-0.07(0.104)
GRN abc	19.25(1.070)	20.34(1.370)	18.14(1.170)
HEAD abc	0.89(0.068)	0.95(0.060)	0.93(0.061)
INOW abc	2.96(0.055)	3.01(0.077)	2.89(0.065)

* All data log(base e) transformed.
\# Letters in brackets indicate no differences between adjusted means.

Three points can be emphasized in the comparison of the two sets of data, that are of interest to later discussion: SNOL is now significantly different between all populations; UPJL is significantly greater in the Grant Lake population, but does not differ significantly between either Bear Lake or Caycuse; Caycuse has significantly longer rakers than either Bear or Grant lakes.

The results of the heritability analysis are given in Table 8. All characters, in all populations, had significant heritabilities ($p < 0.05$) although the average heritability for each population was low, suggesting there is only a moderate amount of additive genetic variance within each population. The estimate of H^2 for GRN is lower than that reported elsewhere ($h^2 = 0.58$, 21°C., Hagen 1973). Comparisons of H^2 values across populations which have been reared under different conditions, are in general of little value, as h^2 is specific to population and environment (Falconer 1981). All genetic components are influenced by gene frequencies and therefore are likely to vary between populations as a result of selection and stochastic forces (Falconer 1981). In addition the component of environmental variance depends on the conditions under which the progeny were raised (a constant environment tends to increase heritability). It is for this reason we seek to reduce the effects of common environment in estimating H^2. Progeny within families reared together, under constant environment, tend to be more similar than those reared apart, which inflates estimates of S^2a, and in turn inflates the value of H^2 obtained.

Table 8. Heritabilities for the three representative morphotypes. The standard error of the estimate is give in brackets.

Table 8. Heritabilities for the three representative morphotypes. Estimates are based on family size weighted intraclass correlations among full sibs.

	Population		
Character	Bear Lake	Caycuse	Grant Lake
HEAL	.2173***	.2155**	.1436**
	(.1243)	(.1375)	(.1197)
SNOL	.1624***	.2614**	.1676**
	(.0896)	(.1534)	(.1006)
EYED	.3229***	.2377**	.7957***
	(.1381)	(.1460)	(.2112)
UPJL	.1489**	.2170**	.3822***
	(.0859)	(.1393)	(.1554)
GRL	.1824***	.6777***	.1320*
	(.0926)	(.2420)	(.0898)
HEAD	.1263**	.1922**	.2566***
	(.0798)	(.1310)	(.1254)
INOW	.4570***	.4122***	.4544***
	(.1705)	(.1946)	(.1699)
GRN	.1438**	.2210**	.3577***
	(.0833)	(.1406)	(.1500)
$\bar{x}$	.22	.30	.34
N	12,357	10,176	14,292

Note: *** ($p < .0001$), ** ($p < .001$), *($p < .01$)

Estimates of heritability may change drastically across environments even if the expressed phenotypic variance remains constant (Hartl 1979). Hagen (1973) found the heritability of lateral plates in the threespine stickleback to decrease from 0.83 to 0.5 with an increase of 4°C. Obviously then, heritabilities depend strongly on population and circumstance. Nevertheless, the results presented here indicate that a significant proportion of the phenotypic variance expressed within populations, arises as a result of variance among genotypes.

Tables 9, 10 and 11 give the genetic, phenotypic and environmental correlations between characters for each population. Many of the genetic correlations within populations are reasonably strong ($0.3 \leq |rG| \leq 0.9$), however the average correlation is much lower as a result of the reduced covariance term for GRN with other characters. In all instances the genetic correlations are greater than the environmental correlations, suggesting the latter are largely residual (Leamy 1977). If the genetic correlations are moderate to high, and the phenotypic correlations are moderate, r(G) may be considered to contribute more to r(P) than does r(E) (Pirchner 1969). This conclusion is supported by the Spearman rank-order correlation coefficients (Table 12) between elements of the three matrices. In the case of both Caycuse and Grant Lake, the rank-order correlations between r(G) and r(P) exceed those for r(E) and r(P). The exception is found for Spearman coefficients within Bear Lake, in which the correlation between elements of r(P) and

Table 9. Genetic, phenotypic and environmental correlation matrices for Bear Lake. I = Genetic correlation matrix, II = Phenotypic (above the diagonal) and environmental (below the diagonal) correlation matrices. All variances and covariances were calculated from log (base e) transformed data.

Table 9. Genetic, phenotypic and environmental correlation matrices for Bear Lake. Diagonal of rG matrix contains genetic variance (*E-3) for each character.

	HEAL	SNOL	EYED	UPJL	GRL	HEAD	INOW	GRN
(I)								
HEAL	.3306							
SNOL	.915	.6036						
EYED	.227	.3036	.9170					
UPJL	-.235	-.159	.7646	.7754				
GRL	-.1426	-.6170	-.4304	.359E-1	2.418			
HEAD	-.0907	.3151	.2726	.6046	-.3264	.4346		
INOW	-.1115	.2558	.5535	.7490	-.4282	.8753	2.122	
GRN	.3963E-3	.855E-2	-.129E-1	.120E-1	.185E-1	-.472E-1	-.23E-1	166.46
(II)								
HEAL		.6350	.2536	.4658	.1684	.2171	.623E-2	-.20
SNOL	.598		.1391	.3035	.2294	.2396	.3300	-.44E-2
EYED	.2658	.0923		.4287	.1223	.2811	.2816	.42E-2
UPJL	.622	.3888	.3438		.4015	.4556	.3493	-.26E-2
GRL	.2458	.4054	.1644	.4754		.2815	.0687	-.23E-2
HEAD	.2807	.2274	.2939	.4321	.3917		.3148	-.45E-2
INOW	-.0440	.3856	.1192	.2266	.2877	.1521		-.26E-2
GRN	-.0059	-.0037	.0583	-.0051	-.0063	-.0021	.0049	

Table 10. Genetic, phenotypic and environmental correlation matrices for Caycuse. I = Genetic correlation matrix, II = Phenotypic (above the diagonal) and environmental (below the diagonal) correlation matrices. All variances and covariances were calculated from log (base e) transformed data.

Table 10. Genetic, phenotypic and environmental correlations for Caycuse. Diagonal of rG contains the genetic variance (*E-3) for each character.

	HEAL	SNOL	EYED	UPJL	GRL	HEAD	INOW	GRN
(I)								
HEAL	.2834							
SNOL	.7899	1.2860						
EYED	.4823	.4811	.9870					
UPJL	.7243	.3724	.1256	1.5280				
GRL	-.3070	-.1912	.6499	-.5158	9.6420			
HEAD	.7396	.9849	1.0357	.0769	.9761	.4696		
INOW	.5258	-.3455	-.2732	.2983	-.3649	-.0417	1.6940	
GRN	.0455	.0569	.0246	.0228	-.0107	-.0181	-.0095	430.6
(II)								
HEAL		.6350	.2536	.4658	.1684	.2171	.62E-2	-.48E-2
SNOL	.5980		.1391	.3035	.2294	.2396	.3300	-.44E-2
EYED	.2658	.0923		.4287	.1223	.2811	.2816	.0416
UPJL	.6220	.3888	.3438		.4015	.4556	.3493	-.26E-2
GRL	.2458	.4054	.1644	.4754		.2815	.0687	-.23E-2
HEAD	.2807	.2274	.2939	.4321	.3917		.3148	-.45E-2
INOW	-.0440	.3856	.1192	.2266	.2877	.1521		-.26E-2
GRN	-.0059	-.0036	.0583	-.0051	-.0063	.0021	.0049	

Table 11. Genetic, phenotypic and environmental correlation matrices for Grant Lake. I = Genetic correlation matrix, II = Phenotypic (above the diagonal) and environmental (below the diagonal) correlation matrices. All variances and covariances were calculated from log (base e) transformed data.

Table 11. Genetic, phenotypic, and environmental correlations for Grant Lake. The digonal of rG contains genetic variances for each character.

	HEAL	SNOL	EYED	UPJL	GRL	HEAD	INOW	GRN
(I)								
HEAL	.3942							
SNOL	.8137	.6534						
EYED	.6965	.2621	2.208					
UPJL	.8409	.4626	.8446	2.474				
GRL	.2106	-.2101	-.1007	-.3129	1.425			
HEAD	.2323	.3049	.4836	.3907	-.8535	1.695		
INOW	-.0343	-.08678	.6279	.5797	-.6632	.7499	1.735	
GRN	.0283	.0221	.01326	.0278	.0325	-.0252	-.0111	500.4
(II)								
HEAL		.5153	.3535	.4748	.3590	.2566	.1620	.41E-2
SNOL	.4576		.2421	.4915	.3995	.2245	.1908	-.41E-2
EYED	.2794	.3552		.5352	.2073	.2495	.3595	-.47E-2
UPJL	.3806	.5221	.1952		.3867	.2344	.3148	-.29E-2
GRL	.3829	.5068	.5699	.6241		.0702	-.0081	-.82E-2
HEAD	.2657	.1934	.0797	.1654	.2830		.2308	-.67E-2
INOW	.2475	.2476	-.0547	.1262	.2247	-.0398		-.41E-2
GRN	-.0031	-.0130	-.0255	-.0210	-.0205	.0014	.0008	

r(E) exceeds that for r(P) and r(G). The latter result may be a consequence of Bear Lake having the lowest estimates of H^2. If the phenotypic correlation is expressed as:

$$r(P) = r(G) * H^2x * H^2y + r(E) * [(1 - H^2x)(1 - H^2y)]^{.5}$$

(Pirchner 1969), where H^2x and H^2y are the heritabilities of characters 'x' and 'y' respectively, the compound nature of r(P) becomes evident. In addition it is apparent that r(P) also

Table 12. Spearman rank correlations between the elements of the genetic, environmental and phenotypic correlation matrices.

		r(G)	r(E)	r(P)
Bear Lake	r(G)	1.0		
	r(E)	0.0071	1.0	
	r(P)	0.4347	0.8236	1.0
	$\bar{x}$	0.3048	0.2331	0.2156
Caycuse	r(G)	1.0		
	r(E)	-0.2184	1.0	
	r(P)	0.6021	0.5362	1.0
	$\bar{x}$	0.3749	0.2191	0.1777
Grant	r(G)	1.0		
	r(E)	-0.1598	1.0	
	r(P)	0.5742	0.5529	1.0
	$\bar{x}$	0.3544	0.2245	0.2249

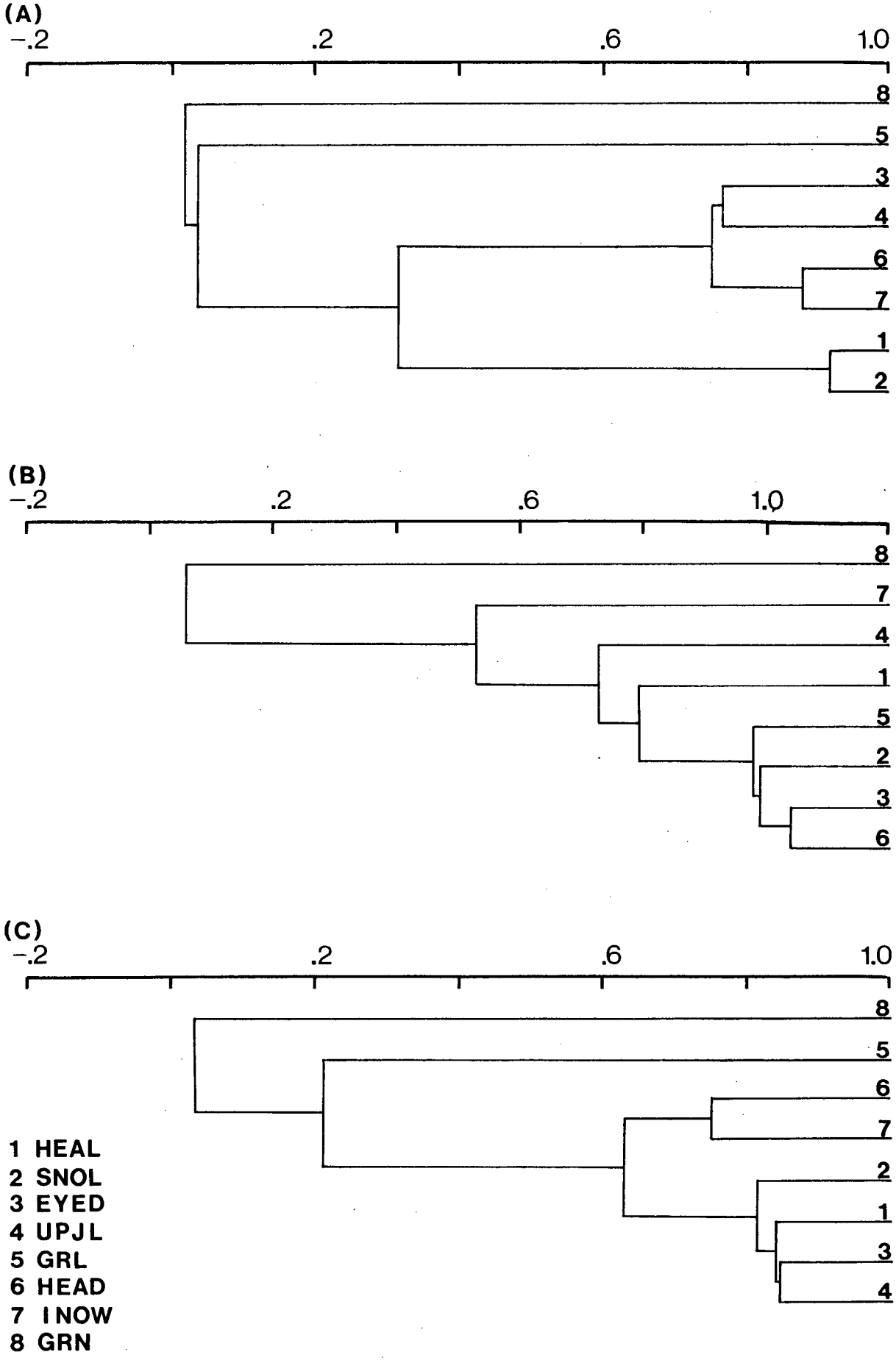
depends on the heritabilities of the two characters. When the heritabilities are small, the environmental component contributes more to r(P), but if the estimates of H^2x and H^2y are imprecise, the relative contributions of r(G) and r(E) remain questionable. This result however does not appear to have effected the overall structure of the correlation matrices

described below, and I have proceeded under the assumption that r(G) contributes significantly to r(P) within Bear Lake.

Figure 6 shows the three dendrogram summaries of character clustering implied by r(G) for each population. Patterns of character clusters are very similar for Grant and Bear lakes. At the level of about r(G) = 0.2, two distinct clusters are evident. The first contains two characters, GRN and GRL, and hence groups the two features of gillraker structure which have been implicated in planktivory (e.g. Kliewer 1970; Lindsey 1981). The second grouping contains the remaining six characters and might be interpreted as a head shape cluster. Interestingly, the character structure of the two groups defined here, is the same as that given by the first two principal components derived from the wild population data (Chapter 1). This supports the conclusion that phenotypic covariance results largely from genotypic covariance.

The character clusters derived from r(G) for the Caycuse progeny are quite different than those described for Grant and Bear lakes. The only similarity to the previous clusters is that the most distant grouping of GRL has been preserved, suggesting it is indeed a distinctly organized character (i.e. is not strongly integrated by pleiotropy, with the other characters measured). GRL has been separated from GRN in the present dendrogram, a result which appears intuitively anamolous. GRN and GRL are both characters associated with a planktivorous existence and one might well expect them to form an integrated character. It is possible that the structure of

Figure 6. Dendrogram summary of character genetic correlation matrices. (A = Bear Lake, B = Caycuse, C = Grant Lake)


(A)
−.2
.2
.6
1.0
8
5
3
4
6
7
1
2
(B)
−.2
.2
.6
1.0
8
7
4
1
5
2
3
6
(C)
−.2
.2
.6
1.0
8
5
6
7
2
1
3
4
1 HEAL
2 SNOL
3 EYED
4 UPJL
5 GRL
6 HEAD
7 INOW
8 GRN

the dendrogram derived from the Caycuse correlation matrix, results from low genetic variances and imprecise estimates of r(G). Bear Lake however, has lower average additive variances for each character than does Caycuse, yet the structure of r(G) for the former population is nearly identical to that of the geographically disparate population - Grant Lake. It seems unlikely that the latter result could arise by chance considering the number of correlations involved. If the structure of r(G) for Caycuse is real, it may indicate that the genome in this population has been in part reorganized.

Selection Gradients

Selection gradients for transitions between population morphologies are given in Table 13. The individual elements βi of each gradient are dimensionless therefore only the relative magnitudes of each βi are of interest. In the calculation of each gradient, z* for the second population was subtracted from z* for the first population, as a consequence, the sign of each βi is a result of the magnitude of the second term and not an indication of divergence on that character.

The βi's for each character averaged across all three populations indicate that the strongest directional selection has operated on HEAL and SNOL, followed by GRN. Average selection intensities for HEAD and UPJL are similar. Relative to these first five characters, much weaker net forces of selection have acted on INOW, EYED and GRL. Net selection distance, B, suggests that the Limnetic-Benthic and

Table 13. Selection gradients for the three representative morphotypes based on the pooled-within variance-covariance matrix.

	Population Transitions		
	Bear/Caycuse	Bear/Grant	Caycuse/Grant
HEAL	2174.64	-219.67	-2394.30
SNOL	-1494.97	188.09	1682.07
EYED	- 190.66	- 15.64	175.02
UPJL	- 771.12	64.02	835.15
GRL	18.50	42.67	61.18
HEAD	902.33	- 76.24	- 978.50
INOW	367.93	- 7.28	- 375.22
GRN	1015.56	-141.03	-1156.59
B	3093.90	259.40	3424.80

Intermediate-Limnetic populations are separated by the greatest selection distance, while the Intermediate-Benthic populations are separated by the shortest selection distance.

Discussion

The relatively low heritabilities observed for the characters scored, suggests there is little additive variance with which ecologically significant characters may respond to selection. This paradox is not uncommon in evolutionary ecology, and has in the past led to tenuous inference of adaptive significance. It is often assumed that characters with low heritabilities, but which are directly related to fitness, lie at equilibrium (an adaptive peak), and that their mean fitness is no longer increasing (Futuyma 1979). However it is equally likely that additive variance was reduced by a founder event (i.e. is the historical consequence of a population

bottleneck). If freshwater populations of Gasterosteus are post-glacial derivatives of an anadromous marine form, as is generally assumed (Bell 1976; Wootton 1984), then certainly the potential exists for the reduction of variance due to sampling error. Natural selection in turn is thought to modify the patterns of extant variation produced by these invasions (Hagen and McPhail 1970). Recently data from electrophoretic studies of the marine and freshwater forms of Gasterosteus, in British Columbia, have provided empirical support for this model (Withler and McPhail 1984).

If these estimates are reasonable reflections of Va, then freshwater populations may not be capable of response to a novel selective regime should it arise, however the expressed variation may not represent the scope of available additive variance. Large amounts of Va may be preserved as 'hidden variance' in negative genetic correlations (Lande 1975). Theoretical models suggest that stabilizing selection will lead to the fixation of positively correlated traits, but that under a constant regime of stabilizing selection, negative correlations will evolve preserving variance (Lewontin 1964). Their effects on the phenotype cancel producing small deviations from the mean. Perturbations of these complexes will lead to the expression of that variance (Rose 1982). Thus it is possible that the genepools of freshwater Gasterosteus populations have preserved variance in coadapted gene complexes and may still respond to selection following a founder event.

Population founder events (the establishment of daughter

populations from founder individuals), may or may not be followed by incipient isolation (which is required if the daughter population is to emerge as a biological species). Not all ancestral populations are thought to possess the genomic architecture required for the evolution of isolation. Carson and Templeton (1984) distinguish between speciating and non-speciating lineages derived from ancestral populations. Non-speciating lineages may arise from the dispersal (or subdivision) of species which show a propensity to colonize - so called 'weed species'. Such an organism probably possesses a generalist genome (Baker 1965), allowing rapid expansion into novel environments but whose architecture is resistant to change by founder effects (Carson and Templeton 1984). These non-mutable genomes are thought to belong to highly inbred or haploid species, yet very old species with tightly coadapted gene complexes may also be non-mutable (Carson and Templeton 1984). Speciating lineages are thought to arise from less tightly integrated complexes which are broken by a founder event and reorganized under selection, resulting in a shift to a new adaptive peak. Incipient isolation may result as a consequence of the movement between peaks (Templeton 1981). What type of genetic architecture has facilitated the evolution of morphological divergence between freshwater stickleback populations? I believe the estimation of the genetic covariance between characters suggests two alternatives.

In reconstructing the pattern of interpopulation morphological divergence, I have assumed constancy of the pooled

variance/covariance matrix. If this measure of 'G' has been made without error it may indicate that Gasterosteus should be viewed as a weed species. The matrix is characterized by weak correlations between characters, which may facilitate shifts in mean phenotype under antagonistic selection (Lande 1979). This selection operates to change the mean phenotype of two correlated characters against the sign of the correlation. By this definition then, antagonistic selection includes two subsets of selective forces: the first arising from selection for directional change in one of two positively correlated traits; the second arising from selection on one of two negatively correlated traits. Shape change and/or change in other characters associated with shifts in trophic resource use (e.g. GRN) will be promoted by these weaker correlations, allowing a more rapid response to a novel selective regime. Could it be that the extensive freshwater diversity of Gasterosteus results from a generalist genome, characterized by 'loosely' correlated complexes of coadapted genes?

The similarity in structure of the Bear and Grant Lake dendrograms would seem to support the hypothesis of a generalist genome. These populations appear to be responding to different environmental constraints with the same architecture. However their organization contrasts with that of Caycuse which shows a much different pattern of character clusters. If the Caycuse structure is real (i.e. does not arise from error in the measurements of Va) then the Caycuse population must be considered genetically distinct from Bear Lake (the

geographically proximate population) and may be reorganized in comparison to it and Grant Lake (or conversely, they are reorganized with respect to Caycuse). Reorganization would imply a more tightly integrated ancestral architecture which was broken during the founder episode, or possibly altered by selection. Unfortunately, we know little about the types or intensity of selective pressures that would be required to alter character correlations. Short term and long term selection experiments have demonstrated considerable constancy of the variance/covariance matrix over time (Cheung and Parker 1974 ; Leamy and Atchley 1984).

In the face of the results, the second strategy (genomic reorganization) seems somewhat unlikely compared to the first (colonizer genome). If genetic reorganization of the ancestral population has occurred it is unlikely that the pattern of character correlations within Grant and Bear lakes would be so similar. Rather, the results suggest a generalist genome responding to a variety of selective regimes, with weak correlations facilitating shifts in trophic phenotype. The significance of these shifts is examined in the following chapter.

CHAPTER 3

Introduction

Reproductive isolation arising through adaptive divergence of subpopulations, is still thought to be a primary mode of speciation (review in Templeton 1981). Those studies which focus on the historical patterns of population divergence typically attempt to correlate morphological or genetic variability with one or more selective constraints (e.g. Mitter and Futuyma 1979; Findley and Black 1983; Felley 1984); that is, evolutionary histories are reconstructed primarily from inference (Mayr 1983). Such 'adaptationist programs' have been criticized for their inability to properly define the targets of natural selection (Gould and Lewontin 1979) leading to the erection of erroneous histories (Chapter 2). However the explanatory power derived from inferential studies may be increased both by an investigation of the organism's ecology (Clarke 1978) and some knowledge of a particular trait's functional significance (Bock 1980). Recently this approach has allowed the direct measurement of natural selection in populations of Darwin's finches (Boag and Grant 1981). This study and others (e.g. Miles and Ricklefs 1984; Mittlebach 1984; Schluter and Grant 1984) have concentrated on selection for divergence in trophic morphology (e.g. beak size) and its correlation with food type and availability.

In studies of teleost evolution, modifications of trophic morphology are thought to be a common mechanism promoting such

evolutionary phenomena as the explosive radiation of the African Great Lakes cichlids (Greenwood 1984). Almost all occurrences of these "species flocks" involve some alteration of the feeding apparatus (review in Eshelle and Kornfield 1984). Divergence in teleost trophic morphology is not limited to cases involving multiple radiations. In systems containing only a single species pair, interspecific differences in trophic morphology appear to be correlated with resource partitioning (e.g. Lindsey 1981).

In the threespine stickleback species complex, interpopulation morphological variability is evidenced in many characters (review in Bell 1984). In Enos Lake on Vancouver Island, biological speciation (Mayr 1963) is associated with extreme divergence in trophic morphology and this divergence has resulted in almost complete separation of food type exploited by the two species (Bentzen and McPhail 1984). In this chapter I wish to examine the functional significance of divergence in trophic morphology between lake-dwelling populations of Gasterosteus within the Cowichan drainage. Having described the site-specific morphological variability of each population within each lake, and proposed three morphotypes, it is my intent to demonstrate that each morphotype is indeed an ecotype (sensu Turesson 1922).

Materials and Methods

Trophic Morphology

In all the feeding experiments described below I have again used animals representing each of the proposed morphotypes. The representative populations chosen were the same as those used in the genetic study (i.e. the limnetic from Caycuse, the benthic from Grant Lake, and the intermediate from Bear Lake). Hereafter the three populations will be referred to as 'limnetic', 'benthic' and 'intermediate' respectively. Three trophic variables were chosen for studies of functional significance: upper jaw length - length of the premaxilla (UPJL), gill raker number(GRN) and gill raker length (GRL). Upper jaw length is thought to be a surrogate measure of mouth gape and hence should be correlated with particle size in gape-limited predators (Aleev 1969). UPJL is a representative of the head shape cluster defined in Chapter 2, and selection appears to have operated strongly on it, in transitions between population phenotypes. Gill raker architecture, was the second character cluster defined and like UPJL, GRN appears to have been strongly altered by selection. Variation in gillraker morphology has been studied extensively and has been shown to be related to planktivory (Magnuson and Heitz 1971; Wright et al. 1983). Gill raker spacing is thought to be the mechanism affecting particle retention in planktivores. For this reason I sought to identify interpopulation differences in spacing; however due to the small size of these animals, differential

spacing is confounded by measurement error. Consequently gill raker density was estimated as a surrogate measure of spacing. Gill raker number and length were determined for individuals from each population from rakers on the first gill arch (Hubbs and Lagler 1958). The arch was then excised from the opercular cavity and an enlarged tracing made of the outline using a Wild-M5 dissecting scope and camera lucida. Area occupied by the gill rakers was determined by digitizing the tracings. Sheffe's test was used to compare differences in relative area. Gill raker density was expressed as the number of rakers occupying one square millimeter. The results were then plotted against standard length and analyzed by ANCOVA.

Diet of each population was broadly characterized by gut contents of samples taken from each lake in late spring. Prey organisms were classified as benthic or limnetic following Kliewer (1970) and a chi-square contingency test performed for diet and population. Feeding patterns may change with dispersal to different lake areas after breeding but this should accentuate dietary differences between the limnetic and benthic morphs. Sticklebacks taken from Cowichan Lake in midwater trawls during winter seem to be entirely dependent on plankton (Carl 1953).

Gape Experiments

The functional relationship between upper jaw length and maximum gape, was determined by presenting brackishwater amphipods (Eogammarus confervicolus) to each of the three

populations. Several workers have used amphipods in determinations of maximum gape for Gasterosteus (Burko 1973, Larson 1976, Bentzen 1982) thus results from the present experiments are readily comparable to previous studies. Individual fish were held in 20 litre aquaria for 3 days prior to each test and fed amphipods. The bottom of each aquarium was painted a uniform brown and aquaria were separated by beige-coloured partitions. Fish were starved for 24 hours preceding each test in order to standardize hunger. Periods of starvation longer than 24 hours have been shown to influence feeding behaviour in Gasterosteus (Beukema 1968).

Amphipods were anaesthetized with carbonated water and measured with an ocular micrometer. Each amphipod was assigned to a size category based on body length. Body length was defined as the distance from the base of the antennae to the base of the uropods with the body flexed (Bentzen 1982). Sixteen size classes were tested ranging from 1.55mm to 13.17mm; size-class divisions were 0.77mm. Amphipods were allowed to recover fully before introduction to the aquaria.

Three amphipods, one from each of three size-classes, were presented to each fish. Pilot studies revealed an appropriate amphipod size range with which to begin each trial. Fish were allowed one hour in which to ingest the prey; after one hour the amphipods were removed, reaneasthetized and remeasured. Fish were fed to satiation with chopped liver after each trial. Following a further 24 hour period of starvation, the test was rerun and each size-class presented was increased by one

division. This sequence was continued until the fish could no longer ingest the maximum class presented for three consecutive days. Data were analyzed by ANCOVA. In the initial analysis, standard length was treated as the covariate to determine the effects of relative upper jaw length on gape, across populations. This analysis was repeated substituting upper jaw length as the covariate. In this instance significant interpopulation differences in mean amphipod size attained must indicate the contribution of some effect other than upper jaw length.

Amphipod Manipulation Experiments

Since wild fish had been used in the gape experiment I was interested in examining the contribution of behaviour to differences in foraging success. In this series of experiments amphipods from a single size-class were presented to individual fish in a 218 litre aquarium for twenty minutes while the observer scored behaviour through a hole in a black partition. The prey size-class chosen was 4.65mm. This size-class had been ingested by fish as small as 30mm from all populations. Fifteen prey were presented to each fish, as some individuals had been observed to take as many as ten prey items during a twenty minute feeding bout. Fish were held individually in 20 litre aquaria and starved for 24 hours. Individuals were placed in the experimental tank for 15 minutes preceding each run to allow time for acclimation. After 15 minutes the prey were introduced from the top of the tank and recording of the trial began after

the first orientation. Five behaviours were scored:

1. Orientation to a prey item
2. Strike on a prey item
3. End of a successful manipulation
4. End of an unsuccessful manipulation
5. A break in orientation with no strike at the prey.

Each trial lasted twenty minutes and fish never consumed all the prey. All data were collected using an OS-3 event recorder (Observational Systems Inc.).

A two way fixed effects ANOVA was performed on the proportion of foraging success. The proportion of foraging success was defined as the number of successful strikes (i.e. those followed by prey ingestion) divided by the number of strikes. Probability plots (cumulative percent of the distribution vs raw data) indicated that these proportional data had a significantly non-normal distribution. Consequently the data were transformed using the arcsine square-root transform (Sokal and Rohlf 1981). The two factors in the ANOVA were population and upper jaw length. As all the fish in this experiment were capable of taking the size-class of prey presented, I predicted no difference in foraging success between populations, given that the behavioural components to foraging success were approximately constant between populations. A significant effect of population or a significant interaction term would implicate some effect, apart from morphology, in benthic foraging success.

Initially upper jaw length was divided into three levels

based on the following standard lengths: 30-40mm, 40-50mm and 50-60mm. Regression equations obtained from previously collected samples were used to determine upper jaw length. Ten fish were to be run in each cell of the ANOVA, however I was unable to attain complete cells for two size-classes which resulted in an unbalanced, and badly weighted design; therefore the data were analyzed as a two way but with only two levels of upper jaw length. The ANOVA was performed using UBC:GENLIN.

Interpopulation behavioural differences in foraging were examined for two behavioural variables which were thought to be relatively independent of morphology: average successful manipulation time and strike probability. Average successful manipulation time was defined as the total time spent handling prey, which were eventually ingested, divided by the total number of prey ingested. Strike probability was expressed as a proportion of the number of orientations which were followed by a strike. A Kruskall-Wallis one way ANOVA was performed on each variable across populations. This non-parametric test was used as it is less sensitive to outliers than its parametric equivalents.

To investigate the relative contributions of these behaviours to foraging success, in addition to the effect of upper jaw length, each behaviour was entered simultaneously into a multiple regression. The proportion of foraging success was the dependent variable in the model; upper jaw length, average successful manipulation time, and strike probability were treated as the predictor variables. The distributions of

foraging success and strike probability were significantly non-normal; consequently these data were again arcsine square-root transformed. This transformation was successful in normalizing the data for multiple regression.

Limnetic Foraging Trials

Foraging ability on limnetic prey was tested in the laboratory using Artemia salinii as the experimental prey. Particulate feeding teleosts tend to be size-selective and behaviour is a significant component of selectivity (O'Brien 1979). Size-selective predation would tend to obscure the significance of morphology, for this reason only a single size class of Artemia was used. In addition nauplii colour appears to be approximately constant at this stage. Differences in prey colour have been shown to mediate differential attack responses for a variety of teleosts (review in Hyatt, 1979). Lab-reared fish were used in this series of experiments in an attempt to standardize any learned component of interpopulation behavioural differences in limnetic feeding. All fish were initially reared on live Artemia nauplii before switching to a mixture of liver and frozen Artemia ; consequently individual representatives of the three morphotypes were exposed to live shrimp for similar lengths of time. Test fish were chosen at random from the tanks in which they had been raised.

Each test was run in the same 20 litre aquaria as used in the maximum gape experiments. Tanks were scrubbed and refilled with dechlorinated water between each trial to minimize

suspended particulates which might alter foraging success. Fish were held individually for two days prior to each test and fed live Artemia to satiation. Tank temperature was 10 ± 1.0 °C. At this temperature total gut evacuation time is more than 16 hours (Tugendhat 1960). Immediately preceding each test, individuals were starved for 24 hours to standardize hunger. At the beginning of each trial 100 Artemia (5/litre) were presented to each individual. Fish were allowed to feed for one hour after which they were removed and sacrificed. Preservation was in 10% buffered formalin. After the fish had fixed for approximately one week their stomachs were excised between the upper and lower sphincters (Wootton 1976), opened, and the contents flushed out with water, using a micropipette. The number of Artemia per stomach was scored with the aid of a dissecting microscope. Nine morphological measures were made on each fish: STDLEN, HEAL, SNOL, EYED, UPJL, HEAD, INOW, GRN and GRL. Gillraker density was determined from regression of gillraker area on standard length.

Foraging success was expressed as a proportion of the 100 prey taken by each fish. Probability plots indicated these data had a non-normal distribution for each population, hence the data were arcsine square-root transformed (Sokal and Rohlf 1980). This transform was successful in normalizing the data. Interpopulation differences in the proportion of foraging success were examined using a one-way ANOVA. Univariate correlations were made within and among populations for each morphological variable scored, against foraging success. All

morphological variables were log (base e) transformed for all analyses.

Results

Trophic Morphology

Sheffe's test on the adjusted mean areas indicated no differences in gill raker area, thus the benthic and intermediate populations appear to be packing fewer rakers into the same relative space as the limnetic fish. ANCOVA for gill raker density on standard length suggests this is probably the case. Slopes of population regressions were not significantly

Table 14. Summary of ANCOVA results for gillraker density on standard length.

	Population		
	Bear	Caycuse	Grant
Mean raker density	13.49	11.26	11.02
Adjusted mean raker density	14.16	12.03	9.42
(Std. Error)	0.42	0.48	0.49

Note: probability of equal adjusted means = 0.000.

different; however there were significant differences between adjusted mean gill raker density (Table 14). For any given standard length, the intermediate and limnetic individuals have more closely spaced rakers then the benthic type ($p < 0.0001$). However, the intermediate sample also had significantly more

rakers than the limnetic morph ($p < 0.001$). Table 15 summarizes the gut content data for samples recovered by pole-seine in May. Diets of the intermediate and benthic morphs were dominated by chironomids and ostracods. A small number of copepods were found in the stomachs of the intermediate morph, but no planktonic crustacea were found in the stomachs of the benthic form. Diet of the limnetic morph was numerically dominated by limnetic and surface prey, although chironomids and ostracods again contributed to diet composition. The chi-square contingency test showed diet type (limnetic or benthic) to depend significantly on morph ($p < 0.001$).

Maximum Gape

The results of the gape experiments are summarized in Table 16. For both covariates, standard length and upper jaw length, the relationship with maximum amphipod length was curvilinear therefore all data were log (base e) transformed. The slopes of all regressions of amphipod length on standard length proved to be significantly different from zero ($p < 0.05$), but the slopes did not differ among populations. The adjusted mean lengths of ingested amphipods were significantly heterogeneous between populations, after the effect of the covariate had been removed. Pair-wise t-tests indicated no difference between the lengths of amphipods ingested by the intermediate and limnetic morphs ($p > 0.05$); however, both of these mean lengths were significantly less than that achieved by the benthic morph. The strongly linear relation between amphipod size and standard length

Table 15. Gut content data from wild population samples.

Table 15. Summary of gut content data from samples recovered with pole-seines in May 1983. Tabulated values are the pooled number of prey items/stomach for each sample

Item	Population		
	Bear (N = 30)	Caycuse (N = 20)	Grant (N = 30)
Chironomids	103	12	128
Chaoborus			1
Megaloptera(larvae)			1
Megaloptera(adult)			1
Ephemeroptera	4		5
Simulidae(adult)	2		8
Tipulidae(adult)	1		
Unidentified insect (adult)	2	6	3
Unidentified insect (larvae)	4		4
Gasterosteus eggs	6	28	99
Unidentified eggs			10
Ostracods	312	20	433
Hydracarina			1
Nematodes	1		5
Gammaridae	2		
Cladocera		26	
Cyclopoid copepods	2		
Calanoid copepods	2	68	

Table 16. ANCOVA results for amphipod size on standard length and upper jaw length.

Table 16. Summary of ANCOVA results for amphipod size on: (a) standard length (b) upper jaw length.

(A) Standard length.

	Population		
	Bear	Caycuse	Grant
Mean amphipod size	1.6527	1.6205	1.8745
Adjusted mean amphipod size	1.6292	1.5577	1.9437
(Std.Error)	0.0575	0.0694	0.0596

Note: probability of equal adjusted means = 0.0007

(B) Upper jaw length.

	Population		
	Bear	Caycuse	Grant
Mean amphipod size	0.7177	0.7037	0.8140
Adjusted mean amphipod size	0.7278	0.7081	0.8008
(Std.Error)	0.0243	0.0285	0.0244

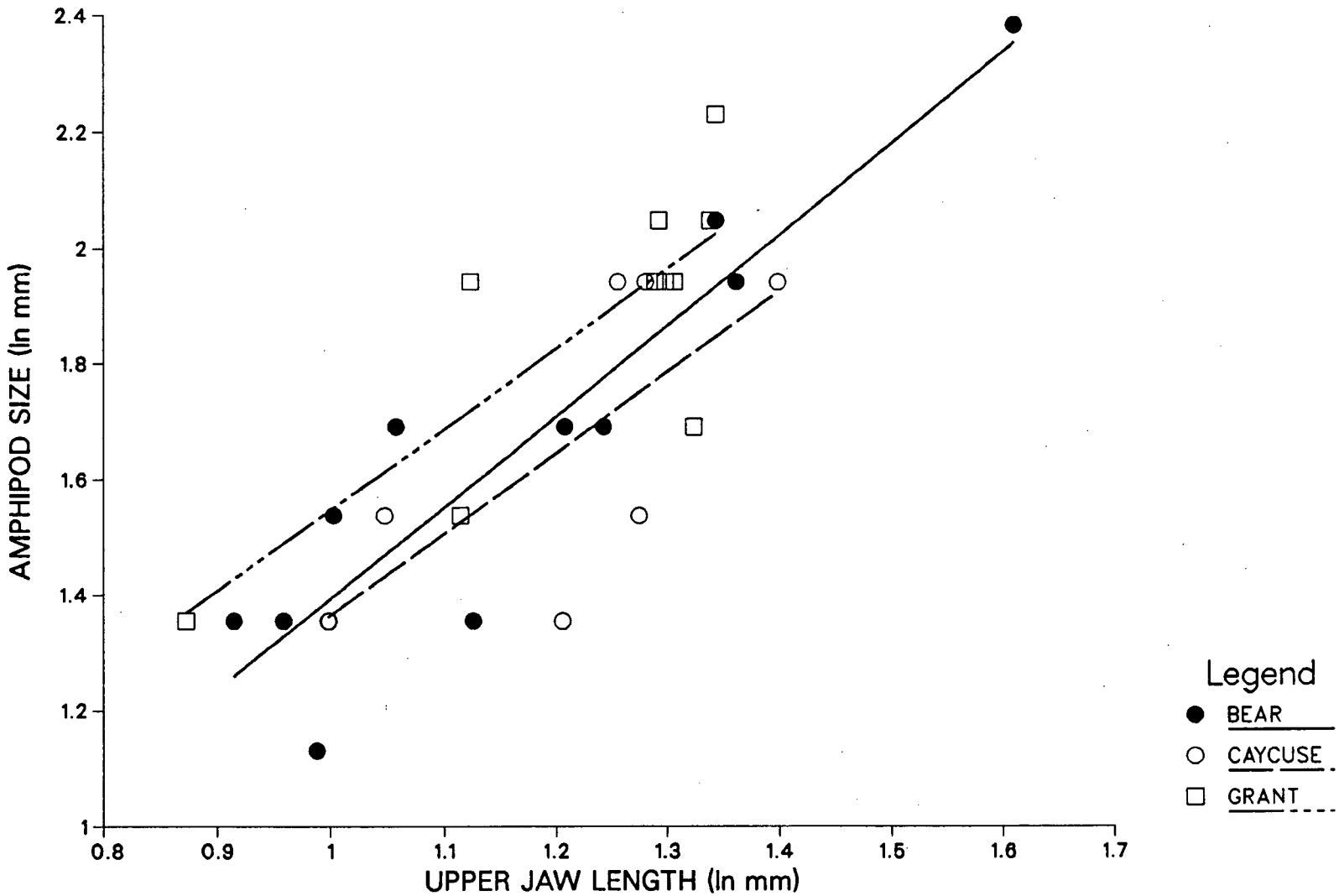
Note: probability of equal adjusted means = 0.04

suggests that the difference in mean size-class attained, arises through increases in mouth gape (upper jaw length) with increased standard length. This conclusion was tested using upper jaw length as the covariate in the ANCOVA. Amphipod size is plotted against upper jaw length in Figure 7. Clearly increased upper jaw length confers an increased maximum gape for all populations. ANCOVA indicated that the slopes of individual population regressions were not significantly different from one another ($p > 0.05$) but the adjusted population means were significantly heterogeneous ($p < 0.05$). Benthic individuals are able to ingest larger prey items than individuals of either the limnetic or intermediate morphs. One should note however that although there is a clear relation between prey size and upper jaw length there are differences between populations that must arise by some mechanism other than differences in upper jaw length. For wild-caught fish, behavioural variability between populations, seems the most obvious source of variable foraging success; therefore I sought to address general behavioural modifications between populations.

Amphipod Manipulation Experiments

The ANOVA indicated no heterogeneity in foraging success among populations ($p > 0.05$) however there were significant differences in variance among the two levels of upper jaw length ($p = 0.0005$). The interaction term (population * upper jaw length) was not significant.. Bartlett's test indicated that all cell variances were homogeneous. This result confirms the

Figure 7. Plot of amphipod size vs UPJL for each morph.


AMPHIPOD SIZE (ln mm)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
UPPER JAW LENGTH (ln mm)
Legend
BEAR
CAYCUSE
GRANT

effect of upper jaw length on foraging success demonstrated in the gape experiments. It remains possible however that

Table 17. Cell means and standard deviations for ANOVA on the proportion of benthic foraging success.

UPJL	Population		
	Bear	Caycuse	Grant
Level 1	0.2099(0.2819)	0.1344(0.2328)	0.2591(0.3127)
Level 2	0.4824(0.2672)	0.5360(0.3191)	0.5372(0.2199)

individual variance in foraging success obscures any extant differences between morph types in foraging behaviour. The within population component of variance is probably inflated by outliers. Standard deviations of mean foraging success were high (Table 17) suggesting individual variation in behaviour does indeed influence foraging success. Aside from this random variation there may be more general differences in foraging behaviour between populations which enhance the effect of upper jaw length in the gape experiments. I therefore attempted to identify patterns of behaviour which might contribute to interpopulation foraging success.

The Kruskall-Wallis ANOVA indicated no differences among populations in probability of strike, but there was significant heterogeneity among populations for average successful manipulation time. The benthic morph appeared to spend less time manipulating the prey before ingestion than the other two morphs. Both of the behavioural variables and upper jaw length were entered into a multiple regression. The independent

variables in the regression are of mixed mode, therefore the data were standardized and the coefficients of regression become Beta weights which are directly comparable (Sokal and Rohlf 1981). The results of this analysis are summarized in Table 18. The overall regression was highly significant ($p = 0.0000$); however only upper jaw length and average successful manipulation time had partial regression coefficients that contributed significantly to the model ($p = 0.0001$). The Beta

Table 18. Summary of multiple regression analysis using arcsine transformed proportions of foraging success as the dependent variable.

Variable	Beta-weight	Std.Error	Significance
Strike probability	0.0829	0.0986	0.4008
Upper jaw length	0.3939	0.0986	0.0001
Average successful manipulation time	0.4106	0.0980	0.0002

Note: multiple R = 0.6117

weights for these variables are very nearly identical. Therefore I suspected that upper jaw length and average successful manipulation time might be strongly correlated; certainly some proportion of manipulation time is expected to result from morphology.

This prediction was tested by correlation for upper jaw length and average successful manipulation time, both within and between populations. In all instances the correlation was negative (Table 19). Pearson's 'r' for data pooled across populations was significant within populations only, the

coefficient attained for the limnetic morph was statistically significant. These results suggest that the contribution of

Table 19. Correlation coefficients for average successful manipulation time and upper jaw length.

Treatment	N	Coefficient
Pooled Populations	70	-0.2981*
Within Populations		
Bear	31	-0.4556
Caycuse	21	-0.5234*
Grant	18	-0.4227

* Significant at $p < 0.05$.

manipulation time to foraging success is largely attributable to jaw morphology. Thus I was unable to identify any general behavioural processes, independent of morphology, that might have produced the differences observed in the gape experiments.

Limnetic Foraging Trials

A summary of limnetic foraging is given for each population in Table 20. Individuals from the benthic population were poor limnetic foragers compared to both the intermediate and limnetic morphs. Sample means were significantly heterogeneous by ANOVA ($p < 0.05$). Sheffe's contrasts indicated no significant differences between the limnetic or intermediate morphs; however, both populations had significantly higher foraging success compared to the benthic population.

Coefficients for all univariate correlations of foraging success with morphology are given in Table 21. None of these

Table 20. Summary of limnetic feeding experiments.

Population	N	Mean Number of Artemia Taken	Mean Proportions of Limnetic Foraging
Bear	30	30.56(21.16)	0.55(0.26)
Caycuse	31	31.93(22.15)	0.56(0.27)
Grant	33	18.03(18.45)	0.36(0.28)

Note: probability of equal means = 0.005.

intrapopulation correlations were significant ($p > 0.05$).

Table 21. Intrapopulation correlations for character and limnetic foraging success.

Character	Bear	Caycuse	Grant
HEAL	-0.1271	-0.1034	-0.0292
SNOL	-0.1710	-0.1826	-0.1445
EYED	-0.1313	0.1362	-0.1980
UPJL	-0.0286	-0.0010	-0.0918
GRL	-0.0887	-0.1921	0.1336
HEAD	-0.2641	-0.0268	0.1100
INOW	-0.2345	-0.1277	0.2757
GRN	-0.1229	-0.0005	-0.2251
GRDENS	0.0563	0.1441	-0.1004

Within each population functional relationships may be obscured by two factors: (a) the limited size range tested for each population and (b) individual behavioural variation. In each population a small number of fish appear to do extremely poorly or extremely well on Artemia. I was hesitant to label these as outliers as they may be extensions of legitimate relationships.

Interpopulation correlation coefficients are given in Table 22. Despite the fact there is only one degree of freedom in this analysis two of the correlations are significant ($p < 0.05$). A chi-square test indicated the probability of finding

Table 22.
Interpopulation correlation coefficients for transformed character and limnetic foraging success.

Character	
HEAL	0.999*
SNOL	0.999*
EYED	0.318
UPJL	-0.454
GRL	0.356
HEAD	-0.033
INOW	-0.069
GRN	0.716
GRDENS	0.865

* Significant at $p < 0.05$.

only two significant correlations, if these arise by chance, was low ($0.05 < p < 0.1$). The bivariate means are plotted for foraging success and adjusted character in Figure 8. In addition to HEAL and SNOL, GRN and GRDENS are strongly positively correlated with limnetic foraging success; the correlation for GRL is in the predicted direction but much weaker. Bivariate means for GRL, GRN and GRDENS with the proportion of limnetic foraging are plotted in Figure 9.

Discussion

Previous investigations have examined the relationship between UPJL and maximum size of particles eaten (Burko 1975; Larson 1976; Bentzen 1982), in all cases the maximum size ingested was a direct consequence of individual gape. Larson (1976) and Bentzen and McPhail(1984) also demonstrated differences in maximum gape between limnetic and benthic

Figure 8. Plots of bivariate means for the proportion of limnetic foraging and adjusted character. Glyphs indicate mean position for each population; black bars indicate one standard error on either side of the mean.

▼ LIMNETIC
● INTERMEDIATE
♦ BENTHIC

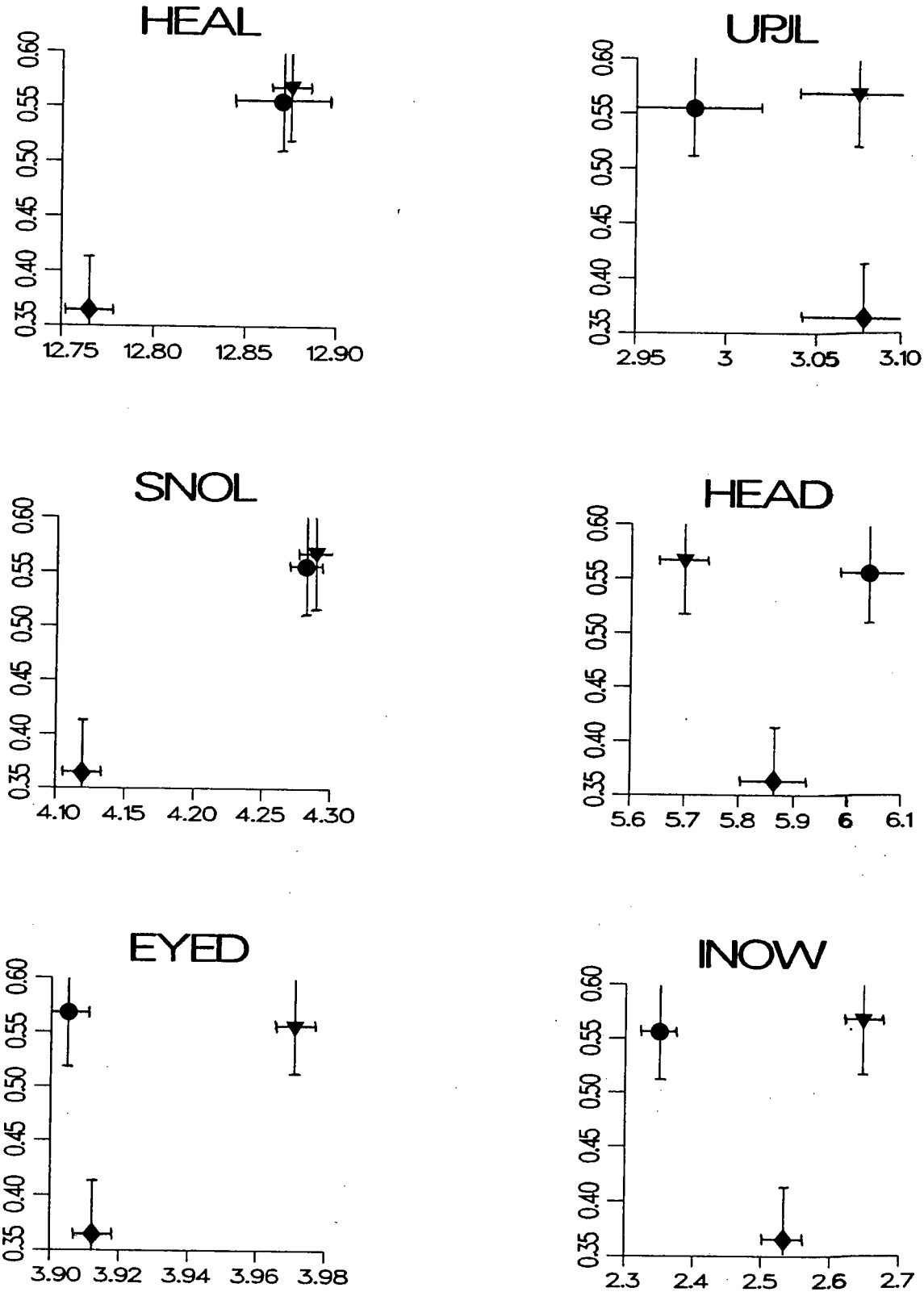
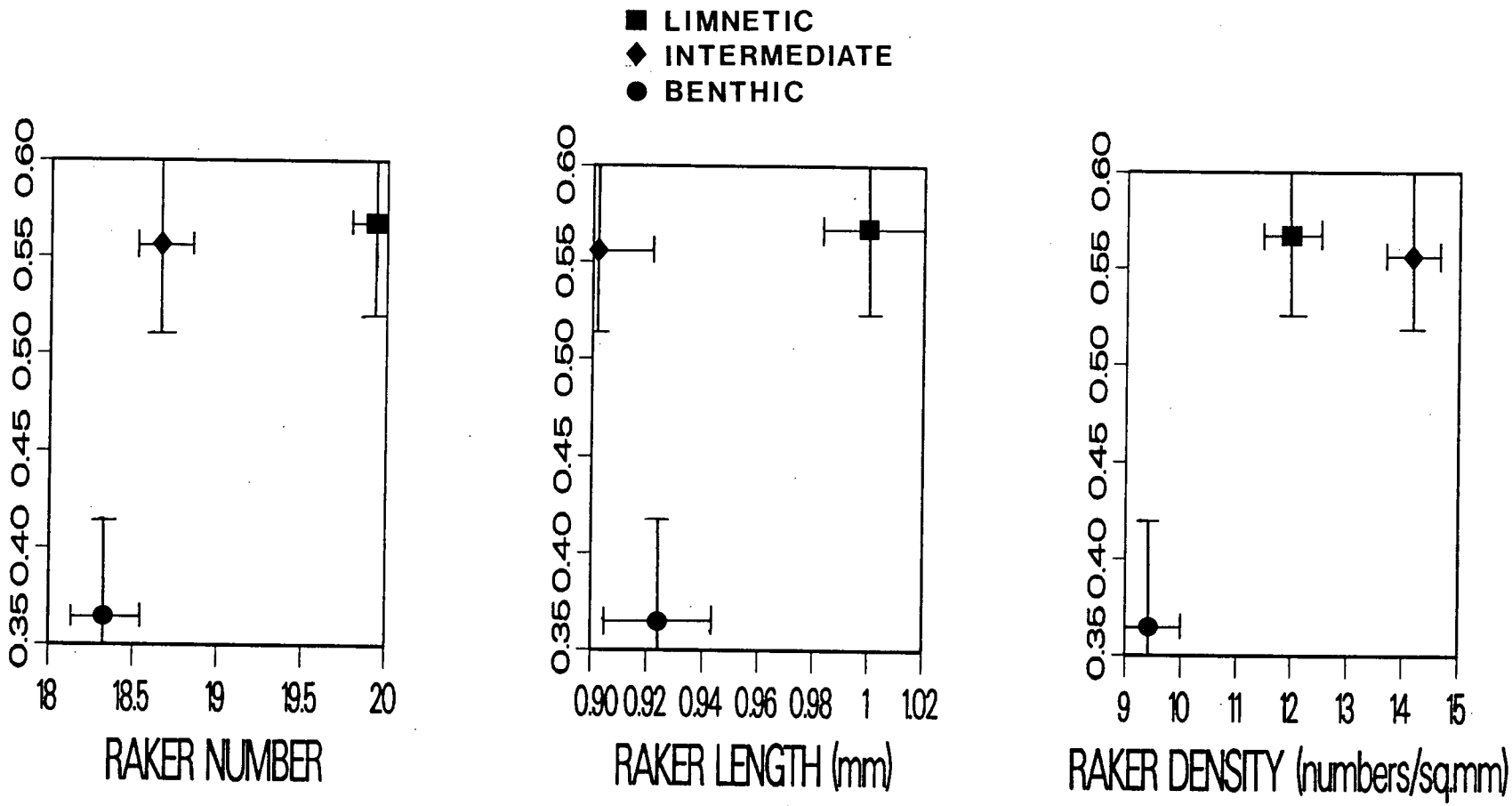


Figure 9. Plots of bivariate means for the proportion of limnetic foraging and GRL, GRN and GRDENS. Glyphs indicate mean position for each population; black bars indicate one standard error on either side of the mean.


LIMNETIC
INTERMEDIATE
BENTHIC
0.35 0.40 0.45 0.50 0.55 0.60
18 18.5 19 19.5 20
RAKER NUMBER
0.35 0.40 0.45 0.50 0.55 0.60
0.90 0.92 0.94 0.96 0.98 1 1.02
RAKER LENGTH (mm)
0.35 0.40 0.45 0.50 0.55 0.60
9 10 11 12 13 14 15
RAKER DENSITY (numbers/sq.mm)

Gasterosteus species pairs. The present study suggests that particle size is a significant selective force operating between populations and hence responsible for ecotypic variation. The benthic with its increased gape is permitted access to a wider range of prey sizes than is the limnetic. In addition increased jaw length results in decreased handling time, which is an energetic advantage and should itself be selectively favoured (Schoener 1971).

The lack of an interpopulation behavioural component to benthic foraging is somewhat surprising as behavioural modification has been shown to be associated with morphological differences in other groups of teleosts (Schultz and Northcote 1972). Bentzen and McPhail (1984) have shown that the limnetic species in Enos Lake, is a poor forager on benthic substrate compared to the benthic species. Lab-reared male limnetics did poorly at sorting prey from the benthic substrate. In their study however, morphological divergence was far more extreme, than that found in the Cowichan drainage, and the limnetic and benthic forms behave as biological species (Ridgeway and McPhail 1984). The Enos Lake species pair is presumed to be the result of a double (or multiple) invasion(s) (McPhail 1984) therefore such behavioural differences may result from historical rather than selective influences.

If behaviour and morphology are tightly correlated it may be unreasonable to attempt to separate their individual contributions to foraging. Indeed a genetic correlation between morphology and behaviour would result in a correlated response

to selection and the possible evolution of a 'trophic character', comprising aspects of both morphology and behaviour. The existence of such a character is supported by the poorer handling ability of F1 hybrids of Enos Lake limnetics and benthics, compared to either of the parental forms (Bentzen and McPhail 1984).

For limnetic foraging, behaviour and morphology are almost certainly tightly correlated. Many features of head morphology contribute to behavioural variation in plankton feeders. Forward positioning of the eyes and increased eye diameter have been shown to increase reactive distance to limnetic prey in Arctic Grayling (Schmidt and O'Brien 1982). As a result of this apparent linkage, it may prove difficult to distinguish the contributions of individual characters. No behavioural component of limnetic feeding was examined in this study and it is possible that the observed differences result entirely from interpopulation behavioural differences. If this is the case however, it would suggest that limnetic behaviour is under genetic control, since the lab-reared fish experienced similar feeding regimes. In this instance, behaviour alone would be the target of selection and again the interpopulation responses are in the predicted direction. The results however, indicate that interpopulation differences in gillraker morphology probably contribute to the superior performance of the intermediate and limnetic morphs on Artemia.

Gillraker morphology may set a lower limit on the size of particle which is retained (Hyatt 1979). The probability that

an individual plankter will escape, after passing into the buccal cavity, is thought to be a function of gillraker retention (Drenner 1977). Hence increased gillraker density is thought to permit a planktonic existence, and numerous studies have drawn a correlation between gillraker architecture and planktivory (e.g. Kliewer 1970; Magnuson and Heitz 1971; Seghers 1975; Wright et al. 1983). The mechanism of gillraker action is still imperfectly understood. In some teleosts, particularly the so-called 'filter feeders' (e.g. Polyodon spathula), gillrakers may act passively as a sieve. In these instances there may be a direct relation between spacing and particle size. For Gasterosteus and other species of particulte feeders however, the role of gillrakers is more complex. Typically particle size retained is somewhat greater than minimum spacing might have allowed (Wright et al. 1983); here again, behaviour seems to modify morphological constraints. By using only one size class of Artemia nauplii one should control for aspects of size-selectivity and be able to identify a 'step' in gillraker composition at which many fewer plankton are retained. Some point must exist at which minimum spacing exceeds the maximum size dimension of the nauplii. Intrapopulation variation may not contain such a step, which would account for the lack of correlation within populations. Interpopulation contrasts however do contain significant breaks in raker morphology and the benthic population with its reduced gillraker density is a significantly poorer planktonic forager than either the intermediate or limnetic populations.

I was unable to determine whether the intermediate morphology of fish from Bear Lake was translated into an intermediate foraging efficiency. This may in part result from the characters tested. Although, in terms of total morphological distance, the intermediate is more closely linked to the benthic morph, UPJL was not significantly different from the limnetic sample. For UPJL then, the success of the intermediate is in the predicted direction. This is also true for limnetic foraging success. Efficiency on Artemia was associated with the higher gillraker densities of the intermediate and limnetic. The gillraker morphology of the intermediate may be a consequence of within generation fluctuating selective pressures. Populations of Gasterosteus move out of littoral regions after breeding, which is often accompanied by a dietary switch to limnetic prey (Gross and Anderson 1983). Although Bear Lake contains an extensive littoral zone, it is dominated by a large pelagic region in which fish no doubt contact plankton. In Bear Lake the breeding season does not last for more than a month and a half, hence the population spends the majority of its life in a pelagic environment. In contrast, Grant Lake contains no appreciable pelagic zone and the population is consistently subject to a benthic environment.

The results of this study indicate that marginal differences in population trophic morphology are sufficient to produce detectable differences in foraging success on a given prey type. The implications of this result are two-fold.

Firstly, the result supports the hypothesis that differences in population trophic morphology, within the Cowichan drainage, are adaptive responses to the primary resource consumed (i.e. a small limnetic prey or a large benthic prey). Secondly, for such variation to be maintained each population must be genetically independent; each population must be considered a race (sensu Dobzhansky 1951). Given genetic independence and an adaptive significance to racial differences, interpopulation variation is clearly ecotypic (Turesson 1922). Geographic distance alone may be responsible for the maintenance of population identity between the benthic and limnetic morphs; however the proximity of the intermediate and limnetic morphs precludes distance as an isolating mechanism. In this instance some degree of habitat selection must be operating to maintain racial distinction, as the breeding seasons of the two forms are concurrent. Once two forms establish divergent habitat choice the framework is established for incipient isolation (Mayr 1963). Hagen (1967) has demonstrated that the freshwater and anadromous forms of Gasterosteus separate during breeding by habitat choice, and Hay and McPhail (1975) have shown that these forms exhibit positive assortative mating, based in part on male choice (McPhail and Hay 1983).

As yet there are no data on assortative mating between ecotypes in the Cowichan drainage, however the investigation of this possibility would be particularly interesting as it may provide insight into the origin of reproductive isolation between the sympatric species pairs. It would be of great

interest to know whether selection maintains racial distinction after secondary contact, or whether habitat selection has led to isolating mechanisms as a byproduct of genetic change. Certainly many laboratory investigations have demonstrated that isolation may arise as a pleiotropic response to morphological shifts under contrasting selective regimes (e.g. Dobzhansky and Pavlovsky 1967; Dijken and Scharloo 1979). Gross changes in morphology however, need not result in reproductive isolation. Sage and Selander (1975) have shown that radiation of trophic morphs may be achieved through polymorphism rather than speciation. A similar conclusion was reached by Turner and Grosse (1980) for the differentiation of Ilyodon. Clearly the next stage of the current research must be the investigation of assortative mating between trophic morphs at some zone of contact, in an attempt to identify the mechanism(s) by which racial integrity in Gasterosteus is maintained.

GENERAL DISCUSSION

Heuts (1947) recognized that natural selection appears to favour distinct complexes of genes controlling plate morphs, in different ecological niches, and that this selection would by definition give rise to adaptive divergence. In the present study I have attempted to demonstrate that selection on trophic morphology may also lead to population divergence. Unfortunately, with no knowledge of the founder population, the term 'divergence' in this instance must describe only the relative difference between population phenotypes (although each population has most likely diverged from a common marine ancestor). The response of trophic phenotype to differences in primary resource type consumed has been demonstrated previously in interspecific comparisons (e.g. Lister 1976; Bentzen and McPhail 1984; Schluter and Grant 1984). The significance of adaptive divergence to speciation in Gasterosteus remains to be demonstrated, certainly conditions appropriate to the establishment of reproductive isolation (e.g. racial integrity) seem to be present in this system. One can only speculate as to whether reproductive isolation would lead to mating barriers, although the latter are thought to be fostered by sexual selection systems (Templeton 1981), some of which have been identified for Gasterosteus (Hagen 1967; Ridgeway and McPhail 1984). Interestingly, sexual selection may be based on trophic features alone (Ratcliffe and Grant 1983).

There are several questions that remain with respect to the variability described in Chapter 1. For example, how

generalized is the interpopulation response of trophic phenotype to primary resource? Within some species populations appear to show multiple solutions to similar selective constraints (Schluter and Grant 1984), it is possible that in a separate river system, the response to selection might be entirely different. In addition the phenotypic response of trophic morphology may be modified by selection on linked character suites.

The extensive intrapopulation variation identified in this study also deserves investigation. Is it due simply to the recombination of the diploid genome each generation, or is it maintained by some selective force? Reimchen (1980b) has suggested that lake-dwelling populations of Gasterosteus may be subject to cryptic intralacustrine environmental differences, which preserve polymorphisms within each population.

Given the divergence of these populations and their apparent individual genetic identity, why has there not been the explosive radiation of freshwater biological species as evident in the cichlids (review in Greenwood 1974). Bell (1976) has suggested that the genetic identity of freshwater populations may be largely independent of adaptive morphology. If this is the case, there must be constraints acting on freshwater systems of Gasterosteus. One possible source of constraint is history. Cichlids are a very old group (Greenwood 1984) and are likely to have been subject to many more transient isolation events in the course of their evolution, primarily those associated with changes in lake level. Although Gasterosteus has experienced

geomorphological events, cichlid populations were probably preserved in refugia and have undergone multiple recontacts. It is unlikely that there were any glacial refugia for freshwater populations of Gasterosteus in British Columbia during the pleistocene. The freshwater evolution of the stickleback therefore appears to be characterized by periods of extinction followed by rederivation from the marine form (Bell 1976).

The second constraint on speciation may be internal. Wootton (1984) feels that the range of variation exhibited by Gasterosteus (including such anomalies as the loss of skeletal elements) represents an 'evolutionary plasticity' under constraint. There are a variety of possible internal constraint mechanisms: genetic (Cheverud 1984); developmental (Alberch 1980); stochastic (Mayr 1983); and ecological (Bell 1976). The present results allow comment on genetic constraints only. The gene complexes underlying trophic morphology do not appear to have been reorganized (Chapter 2), therefore trophic expression is constrained by the extant complexes. As a result, the systems may be forced to respond through trophic polytypism rather than speciation. The relationship between adaptation and speciation remains unclear (Gottlieb 1982), however in this system trophic radiation may be the constrained alternative to speciation. If speciation is so constrained, what is the mechanism promoting the evolution of species pairs (McPhail 1984)?

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