

Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands

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A discriminant analysis was made of 17 morphometric and 10 meristic characters in the freshwater form (leirus) of threespine stickleback (*Gasterosteus aculeatus*) from 16 localities in the Sangan River System on the Queen Charlotte archipelago and in the presumed ancestral form (trachurus) from a nearby locality in marine waters. While multivariate means were significantly different between trachurus and leirus, there was even greater differentiation between the leirus populations found in lakes, ponds, and streams, divergence which was independent of geographical distance between localities. Statistical removal of meristics, removal of body length, log transformation and size standardization of all morphometrics did not substantially alter the discrimination of the populations. Two of the leirus populations that overlap in a narrow zone appear to have achieved reproductive isolation.

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Une analyse discriminante a permis d'étudier 17 caractéristiques morphométriques et 10 caractéristiques méristiques de la forme dulçaquicole (leirus) de l'épinoche à trois épines (*Gasterosteus aculeatus*) dans le bassin de la Sangan, dans l'archipel de la Reine Charlotte, et de la forme présumée ancestrale (trachurus) des eaux marines avoisinantes. Les moyennes multidimensionnelles établissent une différence significative entre la forme trachurus et la forme leirus, mais la différence est encore plus marquée entre les populations de leirus des lacs, des étangs et des ruisseaux, différence indépendante des distances géographiques entre les points d'échantillonnage. L'élimination statistique des caractéristiques méristiques ou de la longueur totale, la transformation logarithmique des données ou la standardisation de la taille de toutes les caractéristiques morphométriques ne réussissent pas à modifier de façon substantielle la discrimination entre les populations. Deux des populations de leirus qui se chevauchent dans une zone restreinte semblent être parvenues à l'isolement génétique.

[Traduit par le journal]

Introduction

Freshwater threespine stickleback (*Gasterosteus aculeatus*) from the Queen Charlotte archipelago, British Columbia, exhibit a range of morphological variation equivalent to that known throughout the circumboreal distribution of the species (Moodie and Reimchen 1973, 1976a). Surveys of gasterosteids (see Bell 1984 for review) have considered disjunct populations over wide geographical areas, yet quantitative data are limited on phenetic diversity among populations in contiguous habitats where gene flow is possible. Such studies on stickleback morphology show univariate clines between habitats (Bell and Richkind 1981) but also differentiation to the level of reproductive isolation (Hagen 1967; Moodie 1972a; McPhail 1984).

We sampled streams, ponds, and lakes in a small watershed, the Sangan River system, on the northeast corner of Graham Island. The multivariate characteristics of these populations are described and compared with a sample of marine stickleback, the presumed ancestor of freshwater populations (Bell 1976).

Study area and methods

The Sangan watershed drains a broad expanse of *Sphagnum* bog and coniferous forest (Fig. 1). The watershed consists basically of three discrete valleys and corresponding streams (Sangan, Skonun, Drizzle) that merge and flow into marine waters of Dixon Entrance. Stream substrate is primarily gravel. Gradients are shallow and headwaters of each major stream are accessible to anadromous species such as coho salmon (*Oncorhynchus kisutch*). Lakes, ponds, and streams are shallow, with maximum depths less than 30, 2, and 2 m, respectively. Their waters are colored a deep brown and have a low pH (4.0–5.5). Aquatic vegetation is sparse: lakes and larger streams have localized stands of *Nuphar*, *Sparganium*, and *Juncus* and smaller streams and ponds have borders of continuous mats of *Sphagnum*.

Collections were made at each of the 5 lakes (>20 ha), at 35 ponds, and at 5 stream localities. Stickleback were found in four lakes, three ponds, and all streams. Solstice Lake (SOL) was the only site sampled from the Sangan branch and is most distant from all other populations. Branta Lake (BR) appears effectively landlocked with an intermittent stream of high gradient. A large beaver dam at the outlet had reduced the flow, preventing the determination of the exact outflow channel. We suspect from the land profile that the stream had flowed into Skonun River. A small sample of stickleback was obtained from Skonun River (SR) immediately below the confluence of Skonun and Drizzle streams and another from 2 km upstream on Skonun Stream (SC). Immediately north of Skonun Lake (SL), a 1-km² *Sphagnum* bog has numerous small ponds (<3 ha) of which three had resident stickleback. Two of these, Big Fish (BF) and Gros (GR) ponds were separated along most of their common border by a *Sphagnum* isthmus (2 m in width) but were connected with a narrow channel. The third pond, Laurel (LA), occurs on the northern edge of the bog that drains north into Skonun Stream.

Drizzle Stream, which forms the secondary branch of the Skonun River, extends southwest into a separate valley where it drains Drizzle Lake (DL). To determine whether there was morphological variation within this lake population, we used samples from three sites on the lake, (i) from the bay where the inlet stream entered the lake (DLI), (ii) from the bay where the outlet creek originated (DLO), and (iii) from the northwestern area of the lake which was most distant from both inlet and outlet bays (DLN). The inlet stream was sampled throughout its length but stickleback were restricted to the lower 500 m. Samples were grouped into an upper (DIU) and lower (DIL) region, the latter occurring within 200 m of the lake. A series of 11 samples was obtained from the outlet creek, the first from the edge of the lake and thereafter at 200- to 300-m intervals downstream. Because stickleback were uncommon, these samples were grouped into an upper region (DA), within 400-m of the lake, and a lower region (DO), from 400 to 2500 m. Further sampling was carried out 6 km downstream but only two stickleback were captured at four separate sites. Much of the length of this stream had no shallow littoral

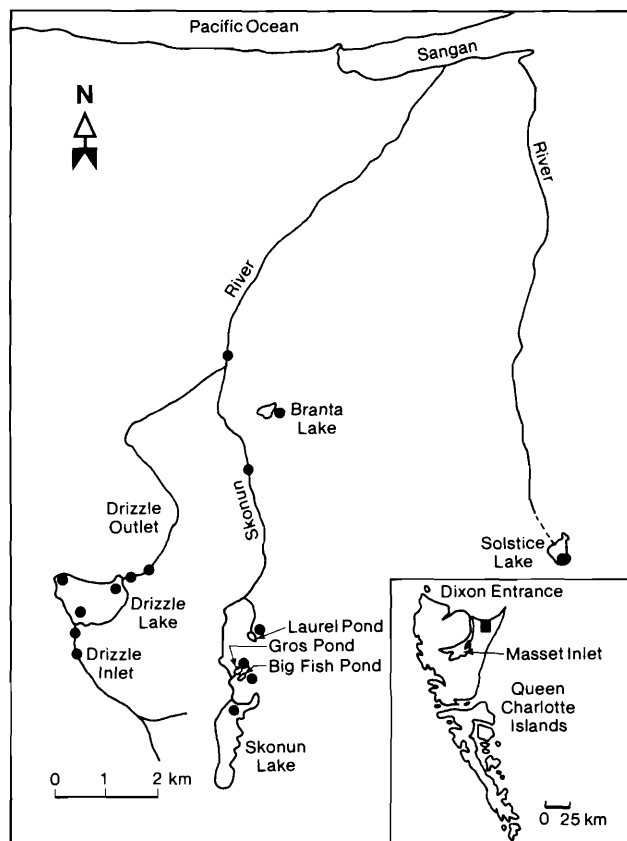


FIG. 1. Study area. Stream channels were drawn from aerial photographs and represent best estimates.

zone and appeared unsuitable habitat for stickleback. Some 20 additional ponds adjacent to the streams were sampled, but no fish populations were found. Cutthroat trout (*Salmo clarki*), a predator on stickleback, was observed in all streams as well as Drizzle Lake and Skonun Lake and was suspected in Solstice Lake. This species, which was not detected during preliminary summer surveys (Moodie and Reimchen 1976a), is more common during winter months when anadromous fish enter the system. To provide a perspective on the morphological relationship with the putative ancestral forms, several samples of marine stickleback were obtained from adjacent marine waters (MA). Only the larger sample from Masset Inlet was analyzed.

All stickleback were collected from April to June with standard minnow traps in freshwater and trawl in marine waters. Fish were preserved in 10% formalin. Twenty-eight characters were scored on each fish, of which 17 represented morphometric traits. Gill rakers were counted following partial staining (alizarin red S). Vertebral counts were made from X-ray plates. Apart from one sample of subadult fish from Drizzle Lake (DLJ), only adult stickleback were used, representing principally reproductive individuals and non-breeding fish of comparable size. Measurements and counts are as follows:

1. Standard length of fish (LENGTH)
2. Maximum body depth (DEPTH): base of first dorsal to venter
3. Length of first dorsal spine (DORSI)
4. Width of first dorsal spine at midpoint of total length (DORSIWDT)
5. Length of second dorsal spine (DORSII)
6. Length of left pelvic spine (LPELV)
7. Length of ventral plate measured from the spine socket posteriorly (VPLATEL)
8. Maximum width of ventral plate posterior to spine insertion (VPLATEW)
9. Height of ascending process (APHGHT) from ventral plate dorsal to tip of process

10. Maximum width of ascending process (APWDTH)
11. Length of pectoral fin from origin to tip of fourth ray from dorsal edge (PECTLNGT)
12. Maximum head length (HEADLNGT)
13. Length of snout (SNOULNLT)
14. Length from snout to anterior edge of first dorsal spine (SNOUDDI)
15. Length from snout to anterior edge of second dorsal spine (SNOUDDII)
16. Width of gape (GAPE)
17. Maximum eye diameter (EYEDIAM)
18. Number of lateral plates on left side of trunk (PLATENO)
19. Number of dorsal fin rays (DRAYS)
20. Number of anal fin rays (ARAYS)
21. Number of principal caudal fin rays (CRAYS)
22. Number of pectoral rays on left side (PRAYS)
23. Lateral crenation of first dorsal spine (CREN): absent (0), moderate (1), and well-developed crenation (2) (see Penczak 1965, Figs. 2g, 2f, 2a for approximate shape)
24. Number of forks on ascending process on left side (APFORK) (see Penczak 1965, Figs. 12a, 12b, 12c, for description).
25. Total number of vertebrae including hypural plate (VERTS)
26. Number of gill rakers on upper arm of first left arch (RAKERSUP)
27. Number of gill rakers on lower arm of first left arch (RAKERSLO)
28. Sex

Two-factor ANOVA (locality, sex) was carried out using log-transformed morphometric values and meristic data. Stepwise discriminant analysis (BMDP7M) (Jenrich and Sampson 1983) was employed to examine the multivariate separation of the populations. The data were tested for homogeneity of dispersion (SPSS-X-MANOVA, SPSS Inc. 1983) which showed heterogeneity in variance (F -approximation = 1.90, $P < 0.001$). While discriminant analyses is sufficiently robust to withstand small discrepancies between dispersion matrices (Hope 1968; Cooley and Lohnes 1971), we have also analyzed the data using log-transformed and size-standardized values which gave closer approximations to normality. The discriminant analysis was performed initially on the combined morphometric (untransformed) and meristic variables and secondly with morphometrics and meristics treated separately. Since total body length and numbers of lateral plates were important population discriminators, further analyses were run in which these variables were excluded.

Adult body size differs within and among localities and, consequently, population differences may be accentuated simply from allometric effects. Additional analyses were carried out using transformations that reduced to the major effects of body size: conversion to percent body length, log transformation, and size standardization. Equality of slopes between localities was tested with analysis of covariance (BMDP IV). Morphometric data were size standardized from the equation

$$\log Y'_{ij} = [\log Y_{ij} - (v_j(\log x_i - \log \bar{x}))]$$

where Y'_{ij} is the adjusted value of character j for individual i , Y_{ij} is the original value, v_j is the average allometric coefficient (regression slope/correlation coefficient), x_i is the standard length of individual i and \bar{x} the mean standard length averaged over all individuals in the study (Wells 1978).

Population centroids (the multivariate population means) for several representative analyses were plotted in three dimensional space and centroids compared with F -statistics. Since population discrimination and centroid positions were similar for raw and transformed data, most results are presented for untransformed data.

Results

Character means and cumulative coefficients of variation for each locality are summarized in Table 1. Most localities in the Sangam watershed contain morphologically distinctive stickleback, including a large melanic form with occasional individ-

TABLE 1. Character means for each stickleback population

CHARACTER	MA	DIU	DIL	DLI	DLN	DLO	DLJ	DA	DO	SR	SC	SK	BF	GR	LA	BR	SOL	CV ^a
LENGTH	60.3	48.4	49.9	79.0	81.7	81.8	50.1	51.6	53.7	51.0	59.2	55.6	67.5	66.9	69.0	61.4	51.6	0.12
DEPTH	13.8	10.7	10.8	15.1	15.6	16.1	9.3	11.7	12.5	12.5	14.5	11.0	16.2	16.3	15.9	12.1	9.7	0.14
DORSI	5.1	4.0	4.0	7.9	8.1	7.9	4.9	4.4	4.6	4.2	4.2	4.2	4.3	4.5	4.2	2.6	2.5	0.15
DORSIWDT	0.8	0.6	0.5	1.0	1.1	1.1	0.5	0.5	0.6	0.6	0.8	0.4	0.8	0.8	0.8	0.5	0.4	0.28
DORSII	5.5	4.6	4.5	8.5	9.0	8.7	5.7	4.9	5.1	4.8	4.8	4.6	5.0	5.3	5.0	3.3	3.0	0.15
LPELV	9.2	7.2	7.1	13.0	13.4	13.1	8.5	7.7	7.9	7.2	7.0	7.1	7.9	8.4	7.8	4.4	4.5	0.12
VPLATEL	10.0	7.3	7.3	11.8	12.1	12.0	6.9	7.5	8.0	7.9	8.7	6.5	9.3	9.5	8.4	5.5	5.1	0.17
VPLATEW	3.2	2.5	2.5	4.3	4.9	4.6	1.7	3.0	3.4	3.3	4.1	2.1	3.6	3.7	3.7	2.3	2.0	0.26
APHGHT	8.1	6.8	6.6	9.5	9.4	10.4	6.4	7.5	7.9	7.5	8.5	7.0	9.2	9.5	8.6	6.2	5.4	0.14
APWDTH	2.4	1.4	1.3	3.1	3.6	3.2	1.6	1.9	2.1	1.8	1.9	1.4	1.7	2.0	1.6	1.1	1.2	0.24
PECTLNGT	9.1	6.0	6.3	10.8	10.8	11.3	6.8	7.1	7.3	7.1	8.0	7.4	8.3	8.1	8.9	7.6	7.2	0.12
HEADLNGT	16.4	13.6	13.7	21.3	21.7	22.6	13.3	15.1	15.4	14.7	17.3	15.1	18.6	18.4	19.4	16.8	14.3	0.12
SNOUTLNG	5.1	3.8	3.7	6.4	6.5	6.8	3.7	4.2	4.3	4.2	5.3	4.3	5.6	5.5	5.9	4.8	3.9	0.16
SNOUTDI	21.5	17.4	17.9	27.2	28.4	29.1	17.8	19.7	20.3	19.6	22.5	19.9	24.4	24.3	25.5	22.6	19.3	0.12
SNOUTDII	28.4	21.5	22.9	34.0	34.8	35.1	22.1	24.2	24.5	24.2	27.8	24.8	30.8	30.4	31.5	27.9	23.2	0.13
GAPE	3.8	3.1	3.2	5.2	5.2	5.8	3.6	3.9	4.0	4.0	4.9	3.8	5.0	4.6	4.8	4.6	3.6	0.12
EYEDIAM	4.5	3.9	4.1	6.1	6.4	6.4	4.1	4.7	4.8	4.4	5.1	4.8	5.3	5.2	5.3	4.9	4.2	0.12
SCUTENO	33.0	3.5	3.7	5.0	5.1	4.7	4.6	4.2	4.2	4.3	4.2	1.3	3.3	3.4	0.2	0.1	0.5	0.23
DRAYS	12.2	11.3	11.4	12.2	11.9	12.1	11.8	11.0	11.0	11.1	10.9	11.1	11.3	11.0	10.2	10.8	9.7	0.06
ARAYS	9.1	8.1	8.3	9.4	9.5	9.2	9.1	8.1	8.4	7.9	8.3	8.8	8.5	8.4	8.1	8.3	7.5	0.08
CRAYS	12.0	12.0	11.9	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	11.9	0.02
PRAYS	10.0	10.0	10.2	10.0	10.0	10.0	9.9	10.0	10.0	9.9	10.0	10.0	9.9	10.0	9.9	10.1	9.9	0.02
CREN	1.2	0.7	0.3	1.2	1.3	1.1	0.5	0.9	0.6	0.7	1.1	0.6	1.1	1.0	1.1	0.9	1.0	0.70
APFORK	2.0	1.4	1.2	1.5	1.5	1.7	1.5	1.2	1.3	1.5	1.3	1.2	1.1	1.1	1.0	1.0	1.0	0.30
VERTS	32.1	31.7	32.0	33.2	33.3	33.3	33.2	31.9	31.8	31.8	32.2	33.4	33.0	32.7	33.0	33.7	32.8	0.02
RAKERSUP	6.2	5.0	5.4	6.5	6.3	5.9	6.3	5.6	5.8	5.4	5.4	6.1	5.8	5.9	5.6	5.8	5.3	0.13
RAKERSLO	15.1	12.2	12.5	15.0	15.1	15.1	14.6	12.6	12.5	11.7	12.2	13.4	12.7	13.1	12.8	13.0	12.3	0.07
Sample size	29	29	24	30	20	30	15	72	44	12	20	38	39	40	40	30	28	
CV ^b	2.9	4.0	4.9	2.8	3.2	3.0	4.3	3.8	5.1	5.9	3.9	7.3	5.0	4.7	7.9	6.0	3.8	

NOTE: All morphometric measurements (LENGTH to EYEDIAM inclusive) in millimeters. See text for description of acronyms.

^aCoefficient of variation of cumulated data for all groups.

^bTotal coefficient of variation based on character mean within localities.

uals exceeding 100 mm body length in Drizzle Lake (DL), small mottled forms (ca. 50 mm) from all streams, deep-bodied fish from Big Fish (BF), Gros (GR), and Laurel (LA) ponds, and relatively slender forms with short spines in Solstice (SOL) and Branta (BR) lakes (Fig. 2). All populations were low plated (*sensu* Hagen and Gilbertson 1973); of these, Laurel (LA), Branta (BR), and Solstice (SOL) had modes at 0 lateral plates while the remainder had modes from 1 to 5 plates per side. All fish had the standard complement of 3 dorsal, 2 pelvic, and 1 anal spine.

Pigmentation ranged from mottled brown in all streams and ponds, to uniform pale brown in Solstice (SOL), dark brown in Branta (BR) and black with silver countershading in Drizzle Lake (DL). In each locality, breeding males were either dark brown or black: red nuptial coloration on the throat found commonly throughout the species distribution, was rare or absent from all localities.

All characters, except number of caudal rays (CRAYS), number of pectoral rays (PRAYS), and sex ratio (SEX) were variable and showed significant differences among localities ($P < 0.001$, ANOVA). Sexual dimorphism was present (raw and log-transformed data) for body and spine length ($F > M$), PLATENO ($M > F$), ARAYS ($M > F$), CREN ($M > F$), and VERTS ($M > F$) ($P < 0.001$, two-factor ANOVA). Total coefficients of variation, cumulated for each locality, were largely comparable, although there was a marginal increase in coefficients of variation in Laurel Pond (LA) and Skonun Lake (SK). Coefficients show no evidence for major introgression or hybridization between the Drizzle Lake fish and those from the inlet and outlet streams. The lake subsamples (DLI, DLO) taken

from the point of closest contact with the stream forms (DIL, DA) had the same variation as that from the opposite end of the lake where no stream stickleback occurred (DLN). As well, the stream samples that bordered the lake (DIL, DA) had similar variances to those collected at greater distances from the lake (DIU, DO). Examination of the character means for each pair of stream samples further indicates no general shifts towards the lake form as would be predicted from clinal variation or hybridization.

Discriminant analysis on untransformed data separated the majority of populations. Of the 28 characters employed in this study, PLATENO, LPELV, LENGTH, and DEPTH were identified as exerting the major effects (Table 2). Canonical axes I, II, and III accounted for 75, 13, and 5% of the variance, respectively. Plots of the locality centroids (Fig. 3) show marine stickleback (MA) maximally separated from all freshwater localities on axis I with PLATENO as the major distinguishing variable. Axis II separates most of the freshwater localities principally on differences in LPELV and DORSIWDT. Centroids for Drizzle Lake subsamples and those from the adjacent stream show no trends for morphological intermediacy in the two areas of overlap (DLI and DIL; DLO and DA). The subadult Drizzle Lake stickleback (DLJ), which were the same size as adult fish from the adjacent stream (DA), were positioned intermediate between the lake adults (DLO) and stream form (DA) on the second axis.

Classification of each fish using Mahalanobis generalized distance (D^2) to locality centroids produced 75% correct identifications (Table 3). All marine fish (MA), Branta Lake (BR), and Drizzle Lake subadults (DLJ) were correctly placed. Of the

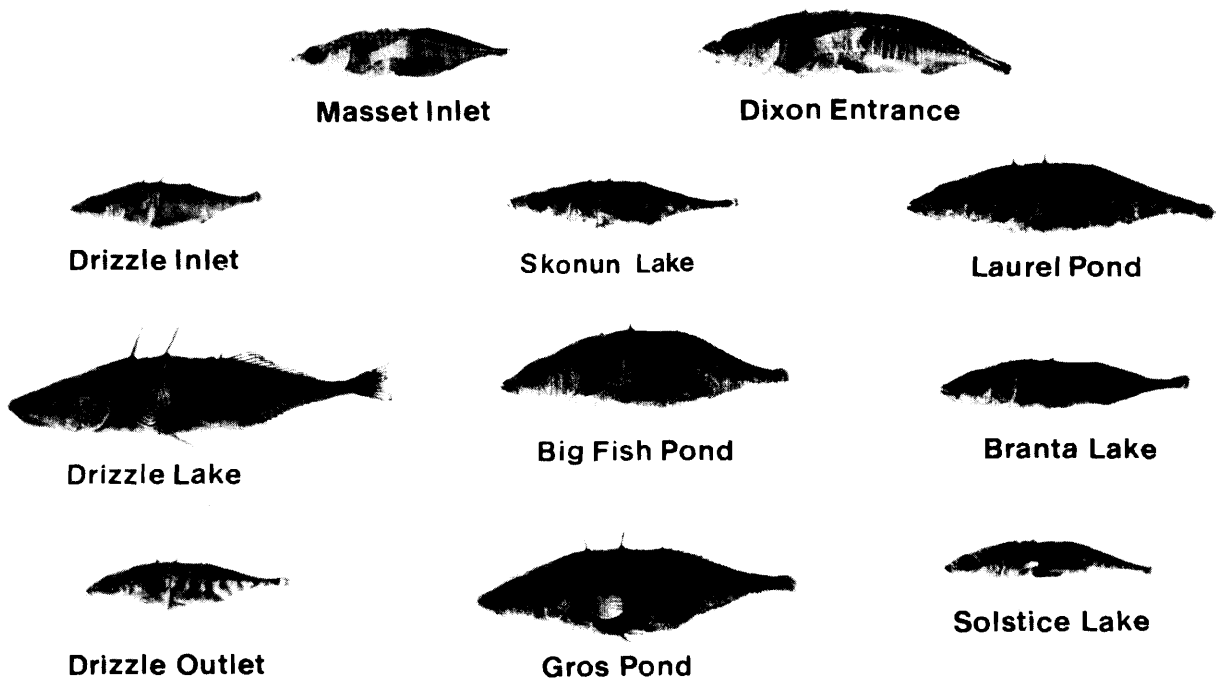


FIG. 2. Representative specimens of adult *Gasterosteus aculeatus* showing differences in total body length, body depth, and spine length. A marine specimen from Dixon Entrance is included for comparison with Masset Inlet.

TABLE 2. *F*-statistic and canonical coefficients for axes I, II, and III. All localities and all variables included; see Fig. 2 for centroid plot

Character	<i>F</i> -statistic	CV1	CV2	CV3
SCUTENO	1377.65	-0.96	0.13	0.07
LPELV	507.46	-0.00	-1.14	-0.40
LENGTH	280.55	0.10	0.01	0.31
DEPTH	205.20	-0.07	0.39	-0.16
VPLATEW	147.71	-0.25	0.03	-0.88
GAPE	113.86	0.20	-0.37	0.67
PECTLNGT	92.81	-0.34	0.08	0.55
EYEDIAM	77.75	0.45	-0.25	-0.65
DRAYS	67.09	-0.06	-0.33	-0.17
APWDTH	59.28	0.60	-0.51	-0.30
DORSIWDT	52.92	-0.60	-0.97	-0.35
VPLATEL	47.99	-0.28	-0.02	-0.29
RAKERSLO	43.94	0.01	-0.26	0.22
HEADLNGT	40.63	-0.06	0.12	-0.18
APHGHT	37.82	-0.02	0.03	-0.27
Eigenvalue		49.18	8.25	3.81
Cumulative dispersion (%)		75.07	87.65	93.47

80 adult fish from Drizzle Lake (DL), 27 were misclassified, but each of these was placed in one of the remaining lake subsamples (DLI, DLO, DLN). None were placed in the adjacent inlet and outlet samples. There was considerable misclassification of stream forms, which were generally placed in another stream sample. A single individual from Solstice (SOL) was misclassified into Branta (BR).

PLATENO, which contributed the main effect on the first axis, was removed and the data were reanalyzed to determine whether marine fish could still be distinguished from freshwater populations. The MA centroid was now positioned within the cluster of leirus populations but all marine fish were correctly classified and no fish from other localities were mis-

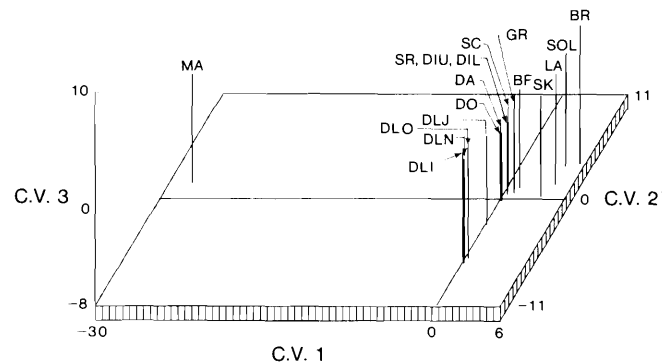


FIG. 3. Three-dimensional plot of population centroids from marine and freshwater localities. See text for acronyms. Canonical coefficients in Table 2 and classification matrix in Table 3.

placed into this group. Log transformation of morphometric variables and removal of all meristics produced centroid plots similar to untransformed data with long pectoral length and small eye diameter distinguishing the marine fish from other populations (100% correct classification). Examination of *F*-matrices ($df = 16, 513$) indicate that marine fish are most similar to Skonun River stickleback ($F = 20.1$) and most dissimilar to Branta Lake ($F = 96.9$) and Solstice Lake ($F = 72.5$).

In subsequent analyses, marine fish were removed and the following population subsamples were grouped (DL = DLI + DLN + DLO, DI = DIU + DIL, DO = DA + DO, SR = SC + SR). LPELV, LENGTH, DEPTH, and PLATENO were the major discriminating characters in these populations (Table 4). Axes I, II, and III accounted for 89% of the variation. Centroid positions (Fig. 4) showed a compact grouping of all stream forms (DI, DO, SR). Drizzle Lake (DL) was maximally separated from Branta Lake (BR), Solstice Lake (SOL), and Laurel

TABLE 3. Identification success from discriminant analysis. All samples treated separately and all variables included

Locality	N	% correct classification	Locality to which individuals were misclassified
MA	29	100.0	
DIU	29	69.0	DIL(8), DA(1)
DIL	24	54.2	DIU(10), DO(1)
DLI	30	56.7	DLO(7), DLN(6)
DLN	20	70.0	DLO(1), DLI(5)
DLO	30	66.7	DLI(4), DLN(6)
DLJ	15	100.0	
DA	72	59.7	DIU(2), DIL(1), DLJ(1), DO(18), GR(1), SR(6)
DO	44	54.5	DIL(1), DLN(2), DLJ(1), DA(12), SC(2), SR(2)
SR	12	66.7	DA(1), DO(1), SC(2)
SC	20	80.0	DO(1), GR(1), SR(2)
SK	38	92.1	DLJ(1), BF(1), LA(1)
BF	39	74.4	DIU(1), DA(1), GR(5), LA(2), SK(1)
GR	40	80.0	DIL(1), BF(6), LA(1)
LA	40	85.0	SK(3), BF(1), GR(2)
BR	30	100.0	
SOL	28	96.4	BR(1)
Total	540	75.2	

NOTE: Values in parentheses indicate numbers of individuals misclassified. N = sample size.

TABLE 4. Canonical coefficients for axes I, II, and III, Masset Inlet excluded; see Fig. 4

Character	F-statistic	CV1	CV2	CV3
LPELV	288.79	-0.77	0.17	0.33
LENGTH	178.97	0.10	0.29	0.01
DEPTH	152.95	0.31	-0.30	0.75
SCUTENO	119.43	-0.30	-0.41	0.10
VPLATEW	99.24	-0.20	-0.79	-0.90
PECTLNGT	83.20	-0.00	0.32	-0.64
GAPE	71.43	-0.27	0.62	0.48
EYEDIAM	62.65	-0.16	-0.16	-1.10
DRAYS	56.30	-0.41	-0.08	-0.03
HEADLNGT	51.05	0.04	-0.26	0.13
RAKERSLO	46.87	-0.16	0.26	-0.05
VPLATEL	43.42	-0.22	-0.36	0.15
DORSIWDT	40.50	-1.24	-0.24	0.27
APWDTH	37.98	-0.04	0.27	-0.66
APHGHT	35.90	-0.07	-0.26	0.31
DORSI	33.96	-0.48	-0.05	-0.24
VERTS	32.25	0.15	0.13	0.20
Eigenvalue		10.32	5.28	2.23
Cumulative dispersion (%)		51.40	77.72	88.84

Pond (LA), principally because of its large spines (DORSIWDT, LPELV, DORSI). The three stream samples (DI, DO, SR) are separated from Big Fish Pond (BF), Gros Pond (GR), and Skonun Lake (SK) on axis II by VPLATEW and GAPE. Each of the pond samples (BF, GR, and LA) has high values on axis III and is distinguished by increased body depth and gape and decreased eye diameter. The classification matrix indicates 89.1% correct identifications with this grouping of samples (Table 5). All fish from Drizzle Lake (DL, DLJ) and Branta Lake (BR) were correctly placed. Two fish misidentified from Drizzle Inlet (DI) were placed in Drizzle Outlet (DO). However, 11% of the fish from Drizzle Outlet (DO) were misclassified and placed in Drizzle Inlet (DI), Drizzle Lake (DL), Drizzle Lake subadults (DLJ), Skonun River (SR), and Gros Pond (GR). Fish from Laurel Pond (LA) were misplaced in

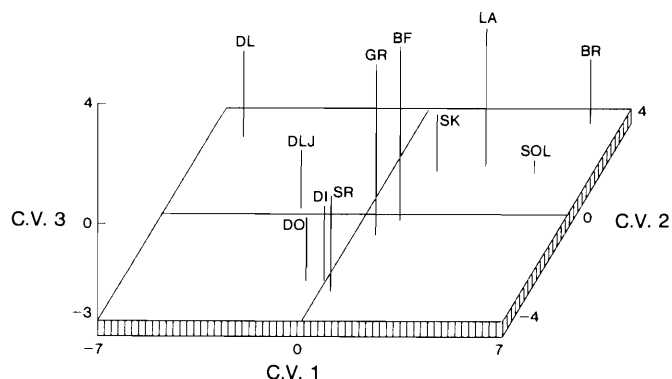


FIG. 4. Three-dimensional plot of population centroids for fresh-water localities. All variables included. See Tables 4 and 5 for canonical coefficients and classification matrix.

either Skonun Lake (SK), Big Fish Pond (BF), or Gros Pond (GR). Skonun River (SR) had the highest misclassifications (28%) with individuals placed in Big Fish Pond (BF), Gros Pond (GR), and Drizzle Outlet (DO). *F*-tests on equality of group means indicate that all pairwise comparisons are highly significant ($P < 0.001$). Minimum *F*-value was 3.93 (df = 17, 484) for the Gros (GR) and Big Fish Pond (BF) comparison.

Transformations of morphometric data did not reduce discrimination of the populations (85.3% correct classification for body ratios and 89.3% correct for log transformation). Major discriminating characters were LPELV, DEPTH, and RAKERSLO. Further removal of all meristic traits did not alter centroid positions and still resulted in high discrimination (83% correct classification). However, using only meristic traits provided limited resolution of the populations (48% correct). RAKERSLO was the major discriminator with Drizzle Lake (DL) having high raker counts and stream forms having low counts.

Size standardization of all morphometric variables to the average body length for the populations (60.7 mm) also did not substantially modify discrimination and centroid positions of the populations. LPELV, LENGTH, and DEPTH accounted for the

TABLE 5. Classification success of discriminant functions. Samples grouped as follows: DI = DIU + DIL, DL = DLI + DLN + DLO, DO = DA + DO, SR = SC + SR; marine population (MA) excluded

Locality	N	% correct classification	Locality to which individuals were misclassified
DI	53	96.2	DO(2)
DL	80	100.0	
DLJ	15	100.0	
DO	116	88.8	DI(3), DL(2), DLJ(2), SR(5), GR(1)
SR	32	71.9	DO(7), BF(1), GR(1)
SK	38	94.7	DLJ(1), BF(1)
BF	39	76.9	GR(7), LA(2)
GR	40	75.0	DI(3), BF(6), LA(1)
LA	40	80.0	SK(4), BF(2), GR(2)
BR	30	100.0	
SOL	28	96.4	BR(1)
Total	511	89.1	

NOTE: Values in parentheses indicate numbers of individuals misclassified. N = sample size.

major effects and yielded 86.8% correct classifications (84% explained variance on first three axes). Yet our analysis for equality of slopes indicated significant ($P < 0.001$) population differences in 11 of the 15 morphometric traits, thus violating the fundamental assumption on size standardization. Population differences in slope would produce false values and possibly distort phenetic differences between populations. Therefore, we restricted the analysis to the meristic traits and the four morphometric variables with homogeneity of slopes (DEPTH, VPLATEL, APHGHT, SNOUTDI). This resulted in loss of discrimination (64% correct classification) yet the fundamental pattern remained. All group means were significantly different (F -test, $P < 0.001$) from each other except for Big Fish Pond (BF) and Gros Pond (GR). Stream forms were distinguished on axis 1 by increased body depth and reduced numbers of anal rays; large lakes were separated from small lakes and ponds on axis 2 by a larger ventral plate and higher numbers of dorsal rays in the former, while ponds were separated from all other habitats by increased body depth and reduced SNOUTDI on axis 3.

Linear map distance along stream channels was measured to examine the possible relationship between geographical distance and morphological divergence. Using Drizzle Inlet Stream (DI) as the focal centroid, no correlation between geographical and discriminant distance (Mahalanobis D^2) was evident ($r = 0.07$, $P > 0.85$). The DI stream stickleback were as close phenetically to Skonun Lake (SK) and Solstice Lake (SOL) as they were to Drizzle Lake (DL) with which they were in contact. When this comparison is restricted to the same habitat type, a relationship is evident since the most geographically distant stream form (SR) is also most phenetically distant from Drizzle Inlet stream (DI).

Discussion

Our survey of streams, ponds, and lakes in the Sangan watershed has yielded a series of morphologically distinct populations of threespine stickleback whose differences are comparable to those observed over broad geographical areas. Vertebral number is as variable within this small watershed as throughout western Europe (Gross 1977). Multivariate analyses indicates that total body length and depth, length of pelvic spines, width of ventral plate, and numbers of lateral plates were generally selected as the most discriminating characters

for distinguishing the populations. Apart from ventral plate width, these are the major traits that have been traditionally used in geographical surveys of gasterosteids (Wootton 1976; Bell 1984). Yet population differences were not restricted to these traits; removal of body length, removal of all meristic traits including lateral plates, and transformation of all morphometric measurements towards size free values did not substantially alter the discrimination or centroid positions of the populations. This suggests that the observed differentiation, even with untransformed data, may be a conservative estimate rather than inflated from statistical artifacts. However, allometric effects remain a possible source of population differences; these effects were reduced but not eliminated by size standardization judged by the marginal but significant centroid displacement of juvenile from adult fish at Drizzle Lake. It appears unlikely that nonheritable effects on meristic or morphometric traits induced from differences in developmental temperature, TDS, or other limnological factors (for example, see Heuts 1949 and Lindsey 1962) could be a significant cause of the observed differences since lake and stream forms collected in a narrow region of habitat overlap (i.e., between Drizzle Lake and inlet stream) had the greatest phenetic separation of all the populations.

Some aspects of this diversity are consistent with habitat correlates in other geographical surveys (Hagen and Gilbertson 1972; Moodie and Reimchen 1976a; Gross 1977, 1978). Body length, number of gill rakers, and number of vertebra were greatest in lakes and lowest in streams, while body depth showed the reciprocal relationship. These trends presumably reflect adaptations to limnetic versus benthic life histories (McPhail 1984).

Other characteristics of the Sangan watershed stickleback differ from the general patterns in the species. Throughout their distribution, stickleback rarely have fewer than three lateral plates per side (Wootton 1976; Reimchen 1983), yet in three localities in the Sangan drainage, lateral plates were absent in the majority of individuals. This is particularly unexpected since trout (*Salmo clarki*) are resident in this watershed (Reimchen and Douglas 1980; Reimchen 1983); these predators are generally found in association with stickleback having six or seven lateral plates (Hagen and Gilbertson 1972, 1973; Moodie et al. 1973; Moodie and Reimchen 1976a). As well as trout, lakes in this region are frequented by nine species of

TABLE 6. Character means of stickleback from Mayer and Drizzle watersheds

Character	Mayer		Drizzle	
	Stream	Lake	Stream	Lake
Mean adult body length (mm)	50.7	84.9	49.1	80.7
Body length/pelvic spine length	6.4	5.3	6.7	6.2
Body length/body depth	4.4	4.6	4.6	5.2
Gill rakers	16.6	21.2	17.4	21.3
Lateral plates	4.7	6.8	3.6	4.9
Total vertebrae	32.5	34.0	31.8	33.3
No. of dorsal rays	10.9	11.6	11.3	12.1
No. of anal rays	8.3	9.4	8.2	9.4
Sample size ^a	71	92	53	80

NOTE: Stream samples collected from within 400 m of the lakes; data on Mayer Lake stickleback extracted from Moodie 1971.

^aSample sizes for Mayer Lake fish differ for each character; those indicated represent minimum numbers.

avian piscivores that regularly forage on stickleback (Reimchen 1980, Reimchen and Douglas 1980, 1984). Absence of lateral plates in these populations exposed to diverse predatory regimes clearly contradicts the generally cited correlation. The virtual absence of red nuptial coloration in breeding males is also exceptional. A similar characteristic found in several stickleback populations from Washington has been interpreted as an adaptation for convergent threat displays with *Novumbra hubbsi*, the Olympic mudminnow (Hagen and Moodie 1979). This species is not found on the Queen Charlotte Islands. It seems unlikely that threat displays with other species are applicable, since in Branta Lake and each of the ponds the lack of red nuptial coloration persists even though stickleback was the only fish species detected. A characteristic of this and adjacent watersheds is the deep staining to the runoff. Light penetration in these waters is predominantly of longer wavelengths, producing a reddish hue on the substrate (personal observations). Possibly, this could reduce epigamic advantages of the nuptial coloration by eliminating the visual contrast between the red throat and background.

Population differences are not directly related to the degree of sympatry and allopatry in any consistent manner. Branta Lake stickleback, which are physically isolated from all other populations, are the most phenetically distinct both from other freshwater populations and from the marine form. Skonun Lake fish and those from connecting streams and ponds show proximity of the centroids, numerous misclassifications, and relatively high variance, which suggest significant gene exchange. In contrast, at Drizzle Lake, where fish are also in contact with stream forms and potentially in contact with other lake populations, the stickleback were morphologically distinct (100% correct classifications). We observed no indication of introgression in the narrow region of overlap between lake and stream habitat, suggesting reproductive isolation. Recent work at this site (Stinson 1983) indicates that both habitat selection and mate preference are in part responsible for the isolation.

The pattern of variation at Drizzle Lake is largely comparable to the stickleback described from Mayer Lake, 40 km to the south of the Sangan (Moodie 1972a). In both lakes, stickleback are very large and black (females have silver counter-shading) while stream forms are small with brown mottling. There is a striking parallelism in character means and trends between the lake and stream form of each watershed (Table 6). Although it is never possible to completely exclude a historical

connection between populations or watersheds, we believe that the convergence in morphology has been independently derived (see also Bell 1974, 1976, and Moodie and Reimchen 1976b). If true, this argues strongly for the effects of selection even at a microphenotypic level and necessarily suggests a very restricted component to sampling and stochastic events in the evolution of these populations.

Freshwater stickleback (*leirus*) are generally considered to be independently derived from the marine populations (*trachurus*) in each coastal watershed (McPhail and Lindsey 1970; Bell 1976). The presence of distinct populations within watersheds as observed by Moodie (1972a, 1972b), McPhail (1984), and in this study implies either multiple invasions and independent origin from *trachurus* or secondary modification of *leirus*. Our analyses using multivariate means place *trachurus* within the centroid scatter of *leirus* populations and marginally closer to stream rather than lake forms. Total phenetic distance from *trachurus* to stream *leirus* is less than the distance between the extremes of *leirus* samples and furthermore less than the distance between parapatric populations such as at Drizzle Lake and surrounding streams. On the assumption that phenetic similarity yields insight into ancestral-descendent relationships of these populations rather than evolutionary convergence, it would seem unnecessary to postulate multiple invasions and polyphyletic origin to explain each distinct lake and pond phenotype in the Sangan watershed. Rather, it is reasonable to suggest an initial evolution of a stream form near the Sangan River mouth where marine forms are assumed to breed, and subsequent dispersal upstream into the three valleys. Each lake and pond form would therefore have been secondarily derived from the stream *leirus* while all stream populations would represent a genetic continuum of the same widely distributed phenotype. Localized geographical isolation in the past may have produced the morphological discontinuities between stream and lake populations but the linear structure of the stream populations combined with the sharp habitat boundaries with ponds and lakes would have produced optimal conditions for parapatric divergence (see Endler 1977).

In several adjacent watersheds where highly divergent stickleback have been found (Reimchen 1984), stratigraphic profiles and radiocarbon dating indicate a maximum age of ca. 9500 years for the lake and pond basins (Warner 1984). If this is also applicable to the Sangan watershed, as seems probable from geological evidence (Sutherland-Brown 1968), the differentiation of the Sangan stickleback would represent two sequential speciation events (*trachurus* to stream *leirus* to lake *leirus*) in 3000 to 5000 generations. Such speciation rates are rapid and comparable to African cichlids (Greenwood 1965). The presence of independently derived species pairs of stickleback in the Mayer drainage (Moodie 1972a) and in several localities in the Vancouver Island region (Larsen 1976, McPhail 1984), all of which appear to be post-Wisconsin in origin, indicates that such speciation events may be more common in stickleback than previously suspected. The cumulative data support the contention (Hagen and McPhail 1970; Bell 1976; McPhail 1984) that *G. aculeatus* is a species complex rather than a single highly polytypic species.

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