

Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipelago on the west coast of Canada

T.E. Reimchen and P. Nosil

Abstract: We investigated defence and trophic morphology of 40 freshwater stickleback (*Gasterosteus aculeatus* L., 1758) populations from the Banks–Estevan archipelago for comparison with the isolated stickleback populations from the nearby Haida Gwaii archipelago. Using 14 size-standardized metric traits and 11 meristic or categorical traits from 1706 individuals (14–54/site), we found that the first principal component (PC1) defined a defence apparatus characterized by high loadings for pelvic spine length, number of forks on the ascending process, number of lateral plates, and overlap between lateral and basal plates. The second component (PC2) defined a trophic apparatus characterized by high loadings for gape length, eye diameter, and body depth. Populations with loss of spines, loss of plates, increased gape, increased body depth, and low gill raker number were most prevalent in ponds and shallow lakes with low conductivity. Most traits were sexually dimorphic, with males exhibiting greater armature and increased benthic trophic adaptations. We observed substantially less morphological variability among Banks–Estevan stickleback populations than among the Haida Gwaii populations and one instance of common ancestry or convergence to the giant black stickleback of Haida Gwaii.

Résumé : Nous avons étudié les structures de défense et la morphologie trophique chez 40 populations d'eau douce de l'épinoche à trois épines (*Gasterosteus aculeatus* L., 1758) de l'archipel de Banks–Estevan pour les comparer à celles des populations isolées de l'archipel voisin Haida Gwaii. À l'étude de 14 caractéristiques métriques standardisées en fonction de la taille et de 11 caractéristiques méristiques ou catégoriques chez 1706 individus (étendue de 14–54/site), nous trouvons que la première composante principale (PC1) définit un appareil de défense caractérisé par des poids élevés pour la longueur de l'épine pelvienne, le nombre de fourches sur le processus ascendant, le nombre de plaques latérales et la superposition des plaques latérales et basales. La seconde composante principale (PC2) définit un appareil trophique caractérisé par des poids élevés pour la longueur de l'ouverture de la bouche, le diamètre de l'oeil et la profondeur du corps. Les populations caractérisées par la perte des épines, la perte des plaques, une grande ouverture de la bouche, une grande profondeur du corps et un petit nombre de branchiospines tendent à prédominer dans les étangs et les lacs peu profonds de faible conductivité. La plupart des caractéristiques sont dimorphes en fonction du sexe: les mâles possèdent une armature plus élaborée et des adaptations trophiques plus importantes pour la vie benthique. Il existe substantiellement moins de variabilité morphologique chez les populations de Banks–Estevan que dans celles de Haida Gwaii; nous avons observé un cas d'ascendance commune ou de convergence avec l'épinoche noire géante de Haida Gwaii.

[Traduit par la Rédaction]

Introduction

The limited species diversity, geographic isolation, and replicated island habitats of oceanic archipelagos offer unique opportunities for investigations of biogeography and evolution. One of the dominant themes to emerge in such investigations is the primacy of selection in structuring morphological and behavioural adaptations among taxa (Reimchen 1994; Baldwin 1997; Grant 1998; Losos et al. 1998; Malhotra and Thorpe 2000; Grant and Grant 2002).

On the west coast of Canada, most biotic communities in the isolated archipelagos became established and diversified following deglaciation during the late Pleistocene and early Holocene. Occasional endemism is present within several taxa of plants, fish, and mammals (Foster 1965; Calder and Taylor 1968; Schofield 1989), and this may have resulted from relict source populations that persisted in nearby glacial refugia on the continental shelf (Warner et al. 1982) or from rapid postglacial differentiation from southern populations that colonized the emergent terrestrial and freshwater habitats. Molecular data, for example, show support for both hypotheses (Gach and Reimchen 1989; Byun et al. 1999; Reimchen and Byun 2005). Whatever the historical context, these endemic taxa exhibit unique morphological attributes that likely represent adaptations to island habitats and distinguish them from continental taxa. An outstanding question is, how geographically widespread and how replicable are associations between ecology and morphology?

A model system that can be used to address this question

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is threespine stickleback, *Gasterosteus aculeatus* L., 1758, because the functional significance of many morphological traits is known and geographic variation in morphology is widespread. Morphological variability in threespine stickleback is common in western North America (Hagen and Gilbertson 1972; Bell 1984; Reimchen et al. 1985; Francis et al. 1986; Schluter and McPhail 1992; McPhail 1993; Bourgeois et al. 1994; Baker et al. 1998; Vamosi 2003) but is particularly accentuated in populations from the *Sphagnum*-dominated lowlands of northeastern Haida Gwaii on the west coast of Canada. These populations display some of the greatest departures from ancestral forms, including examples of body gigantism and major loss of armour (Moodie 1972; Moodie and Reimchen 1976a, 1976b; Reimchen 1980, 1984; Reimchen et al. 1985) as well as highly atypical associations between unarmoured stickleback and dinoflagellates (Reimchen and Buckland-Nicks 1990). This concentration of endemics might be intimately associated with a *Sphagnum* landscape, which is characterized by heavily stained and acidic waters as well as a specific assemblage of salmonid, avian, and macroinvertebrate piscivores (Reimchen 1994). However, long-term isolation in nearby refugia might also contribute (O'Reilly et al. 1993; Reimchen and Byun 2005).

Further understanding of morphological diversity in west coast *Gasterosteus* species can be obtained by examining regions that are geographically distinct from previously studied areas yet exhibit a comparable range of ecological landscapes. Examination of such areas allows tests for replicated trends within and between distinct regions. With this motivation, we sampled stickleback from an unstudied geographical area with a major expanse of *Sphagnum*-dominated habitats. This area occurs on the Banks–Estevan archipelago, 120 km to the east of Haida Gwaii and adjacent to the British Columbia mainland, and allows tests for parallel differentiation because there are multiple islands, each with multiple freshwater lakes.

Based upon previous studies of Haida Gwaii stickleback, a number of predictions can be made. The relatively close concordance between lake habitat and stickleback morphology on Haida Gwaii (Reimchen 1994) suggests that on the Banks–Estevan archipelago, the large dystrophic lakes with predatory fish and avian piscivores will yield evidence of gigantism in stickleback (>80 mm standard length), while shallow bog ponds with no predatory fish and few predatory birds will produce stickleback with loss of spines and complete loss of plates. We also predict that the extent of morphological variation among lake populations within islands will be as great as that among islands with a comparable range of lake habitats. Thus, the current study provides insights into morphological evolution in relation to ongoing studies, particularly with respect to how widespread and replicable the roles of ecology and refugia are (i.e., are habitat-associated and relict forms restricted to Haida Gwaii?).

We also examine a number of other factors that have been less explored in studies of geographic variation of *Gasterosteus* species, including the extent and causes of sexual dimorphism and the causes of divergence in trait means versus variances. Thus, the goals of the present study are multiple and include (i) examination of morphological diversity among stickleback populations from lakes of the Banks–Estevan archipelago, both to assess the extent of divergence

itself and to provide a comparison with divergence observed in dystrophic lakes of Haida Gwaii, (ii) comparison of morphological trends within and among islands and with habitat to identify selective landscapes, (iii) testing for sexual dimorphism in defence and trophic structures, and (iv) comparison of trait means with levels of intra-population trait variance recently described for these populations (Nosil and Reimchen 2005). Collectively, the results assess the general role of ecology and geography in morphological evolution and, in particular, the distribution and evolution of endemics adjacent to the putative late Pleistocene glacial refugia on the continental shelf of central British Columbia (Warner et al. 1982; Reimchen and Byun 2005).

Materials and methods

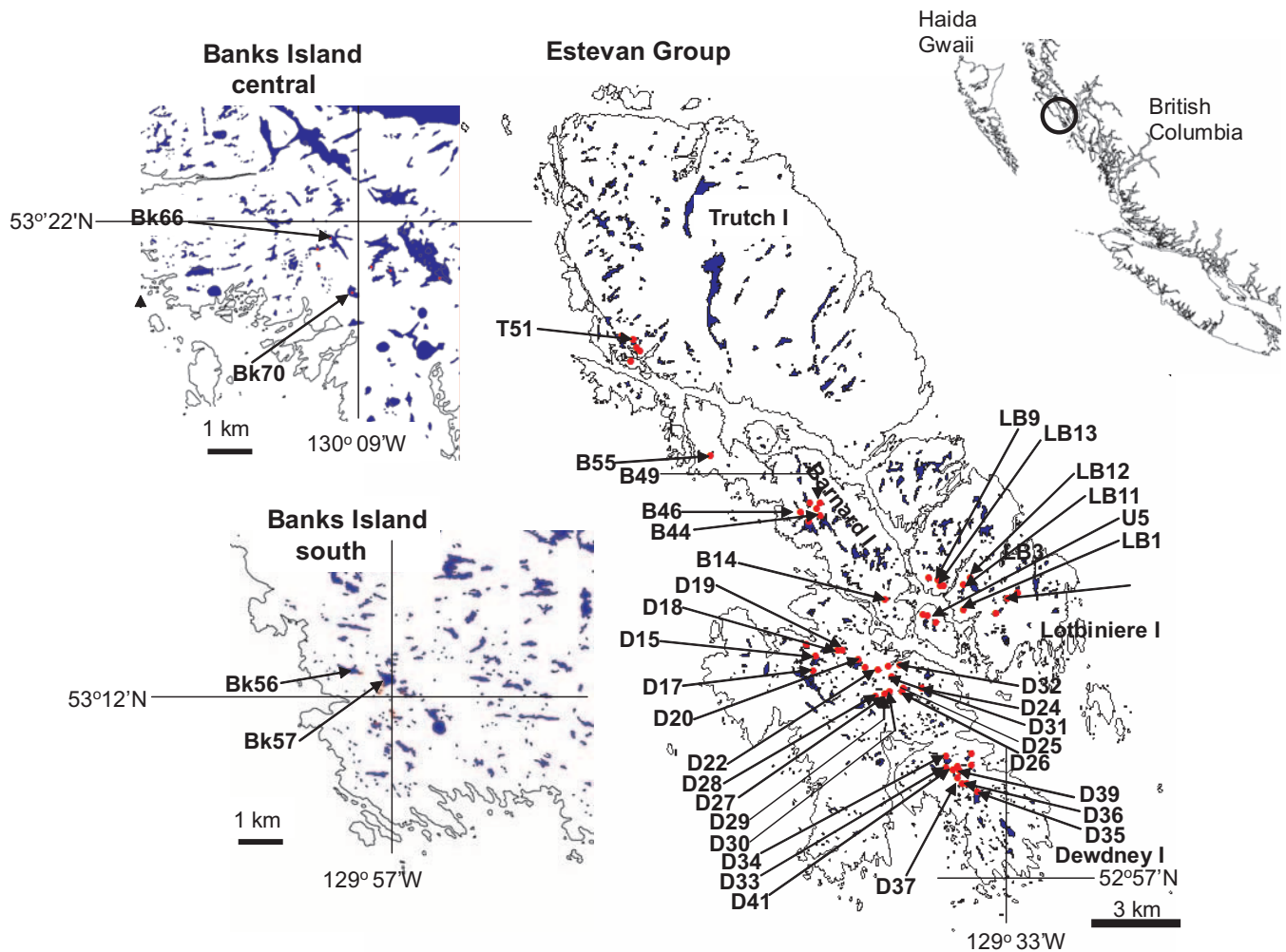
Sampling localities and habitat description

The Banks–Estevan archipelago is immediately adjacent to the mainland mid-coast of British Columbia (Fig. 1). Dewdney Island, on the southern edge of this archipelago, is a combination of minerotrophic *Sphagnum* bog and forest, primarily lodgepole pine (*Pinus contorta* Dougl. ex Loud.), with large numbers of ponds and shallow lakes with clear water and typically with macrophytes including water lily (*Nuphar luteum* (L.) Smith) and buckbean (*Menyanthes trifoliata* L.) (Krajina et al. 1978). Banks Island, the most northerly of the islands, is more densely forested with lodgepole pine, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), while lakes tend to be larger and more dystrophic.

Seventy ponds and lakes were surveyed. Fish were collected with standard G-minnow traps (8 mm diagonal mesh) baited with old cheddar cheese and set overnight in the littoral zones. Samples were preserved in 10% formalin. Forty-two localities contained fish, and threespine stickleback was the most abundant species. At each lake, general biophysical information was recorded, including percent cover of the dominant macrophyte (*N. luteum*), conductivity, and pH. Water samples were taken for spectral analysis (percent transmission at 400 nm). Based on shoreline profile and the horizontal distribution of macrophytes, maximum lake depth was estimated and categorized into broad groups (<1 m, 1–5 m, 5–10 m, >10 m). Lake area was extracted from topographic maps and lake volume was estimated from shoreline profile and area.

In all localities, presence or absence of piscivores was recorded. Macroinvertebrate piscivores such as *Aeshna* (Odonata) and *Dytiscus* (Coleoptera) species were commonly observed or collected and were probably present in all ponds and lakes. Salmonids (*Oncorhynchus* and *Salvelinus* spp.) and cottids (*Cottus* spp.) were detected in several of the large lakes but in none of the ponds and small lakes. Yet in these ponds and lakes without salmonids or cottids, juvenile coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)) were commonly found in outlet streams, indicating that these water bodies were accessible to anadromous fish. On nearby Haida Gwaii, predatory salmonids (*Oncorhynchus clarkii* (Richardson, 1836), *Oncorhynchus mykiss* (Walbaum, 1792), *Salvelinus malma* (Walbaum, 1792)) are frequently absent from small freshwater lakes in summer but enter these during autumn and winter when rainfall, stream flow,

Fig. 1. Map of the study area off the coast of British Columbia. Insets show sampling sites from central Banks Island, south Banks Island, and the Estevan group.



and pond levels are higher (Reimchen 1992a, 1994). We observed one or more avian piscivores, including loons (*Gavia stellata* (Pontoppidan, 1763)), mergansers (*Mergus merganser* L., 1758, *Mergus serrator* L., 1758, and *Lophodytes cucullatus* (L., 1758)), and Belted Kingfisher (*Ceryle alcyon* (L., 1758)), in each of the larger lakes, but these taxa were less predictable in small lakes and ponds. These piscivores are not resident in any particular pond or lake but rather move daily among localities within a broader foraging habitat, so their absence from any particular pond at the time of fish collection is not informative. The best predictors of predator landscape are surface area and depth: shallow ponds are characterized by macroinvertebrate piscivores and small avian piscivores (*L. cucullatus*, *C. alcyon*), whereas large, deeper lakes are characterized by a broad diversity of avian piscivores as well as predatory fish such as salmonids. Based on previous studies on Haida Gwaii (Reimchen 1994) and more limited field observations in this study, we suspect that the proportion of total mortality due to predation could be comparable among the localities.

Using the log-transformed habitat variables (surface area, volume, maximum depth, percent macrophyte cover, conductivity, and water colour) and pH (Table S1¹), we calculated principal component factors of biophysical habitat. The first component (PC1) explained 42% of the variance, with the highest negative loading for percent macrophyte cover (−0.68) and high positive loadings for lake volume (0.96), maximum lake depth (0.92), and lake surface area (0.77). This factor is a proxy for lake morphometry: negative values characterize small, shallow ponds with high macrophyte cover, and high positive values characterize large, deep lakes with steep shores and few macrophytes. The second component (PC2) explained 26% of the variance and had a high negative loading for water colour (−0.61) and high positive loadings for pH (0.74), percent macrophyte cover (0.55), and conductivity (0.53). This factor is a proxy for water chemistry: high negative values describe the stained and low-pH conditions of dystrophic habitats, and high positive values describe clear waters with high conductivity. We used these two factors as general lake descriptors

¹Supplementary data for this article are available on the journal Web site (<http://ejz.nrc.ca>) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5024. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

and tested whether these biophysical indices were correlated with multivariate morphology (see below) using bivariate correlation. Additionally, we tested whether any observed correlations differed among islands using ANCOVA (tests for homogeneity of slopes).

Morphological population differentiation

We scored 14 metric and 11 meristic or categorical traits (Fig. 2) from 42 populations. We excluded two populations because sample sizes were too low (<10), but most samples were large enough that we could score 25 males and 25 females from each locality (see Tables S2 and S3¹ for sample sizes and further details). We scored the following traits: standard length of fish (SL), maximum body depth (BODYD), width of gape (GAPEW), length of gape (GAPEL), length of 1st dorsal spine (DORS1), crenation of the dorsal spine, length of left pelvic spine (LPELV), length of ventral plate (VPLATEL), width of ventral plate (VPLATEW), height of ascending process (ASCPROH), maximum width of ascending process (ASCPROW), length of pectoral fin (PECTFINL), diameter of eye (EYEDIAM), height of 7th lateral plate (PLATEL7), overlap between basal plate of the 1st dorsal spine and plate 4 (OVLAPD1), overlap between basal plate of the 2nd dorsal spine and plate 7 (OVLAPD2), number of lateral plates on left side of trunk (PLATENO), number of dorsal fin rays, number of anal fin rays, number of caudal fin rays, number of pectoral fin rays, number of upper gill rakers (RAKERSUP), number of lower gill rakers (RAKERSLO), number of forks (FORKNO), and sex. Counts of fin rays and gill rakers were made on only 10 fish of each sex for each locality. We recorded any loss (absence) of dorsal or pelvic spines. Evidence of reproductive activity (gravid females, male nuptial color) was noted.

Morphological characters were log-transformed prior to statistical analyses. For all morphometric characters, trait size increased linearly with SL (all $p < 0.001$, bivariate correlation). Consequently, morphometric data were size-standardized (to uniform SL) according to eq. 1:

$$[1] \quad \log y'_{ij} = \log y_{ij} - \beta(\log x_i - \log x)$$

The slopes of the trait size versus SL regression differed among localities for all metric characters (all $p < 0.001$, trait \times SL interaction, ANCOVA) and, consequently, all size standardizations were based upon separate, within-group slopes. Analyses using a common, pooled slope yielded congruent results.

In the majority of cases, meristic values did not increase with increasing SL ($p > 0.05$, bivariate correlation) and therefore were not size-standardized. The single exception was FORKNO, which showed extremely weak, yet statistically significant (at least without adjusting for multiple comparisons), associations with SL ($r = -0.06$, $p = 0.009$). For consistency, size-adjusted values for this trait were used in the principal components analyses described below. However, in the univariate description of this trait, we refer to raw values to allow comparison with other studies. Using the alternative values (i.e., raw versus size-adjusted) for this trait did not affect the overall outcome of any analysis. Sex ratio did not differ among samples ($\chi^2_{44} = 23.85$, $p = 0.99$).

In previous studies of stickleback population differentiation in North America and Europe, several traits, including standard length, number of lateral plates, pelvic spine length, incidence of dorsal and pelvic spine loss, and number of gill rakers, have been important in characterizing population differentiation (Hagen and Gilbertson 1972; Moodie and Reimchen 1976a; Reimchen et al. 1985), so we will consider these traits in more detail.

Multivariate differentiation of the study populations was assessed using principal components analyses (Norusis 1993). These analyses excluded both fin rays and height of the 7th plate because fin rays were relatively invariant and the 7th plate was absent in many individuals. We performed three principal components analyses: (1) one including all the traits but only about half of the fish, as rakers were scored on only half of the fish and overlap was missing on some other fish ("all traits analysis"), (2) one using a slightly larger sample of the fish by excluding overlap and forks as traits ("reduced analysis"), and (3) one including all the fish but excluding overlap, forks, and rakers ("all fish analysis").

These three analyses gave results that were extremely similar. Three lines of evidence illustrate the congruence among data sets. First, for traits that were included in all three analyses, the trait loadings in different analyses were essentially identical (PC1, all $r > 0.97$, all $p < 0.001$; PC2, all $r > 0.90$, all $p < 0.001$; correlations among trait loadings; also see Table 1). Second, individual values for the same PC axis in different analyses were highly correlated (PC1, all $r > 0.96$, all $p < 0.001$; PC2, all $r > 0.98$, all $p < 0.001$; correlations between loadings for the same individual in different analyses). Third, population means for the same PC axis in different analyses were highly correlated (PC1, all $r > 0.97$, all $p < 0.001$; PC2, all $r > 0.93$, all $p < 0.001$; correlation between population means for the same PC axis in different analyses). Consequently, for all further analyses we present only results obtained using the reduced analysis and we stress that using PC axes derived from alternative analyses does not alter our conclusions in any way (notably, the reduced analysis represents a compromise between including more traits and including more individuals).

We used nested ANOVA to test for morphological variation among islands, among populations within islands, and among individuals within populations.

Results

Univariate population differentiation

Morphological traits of stickleback varied both within and among islands (Fig. S1, Tables S2 and S3¹). Maximum body length of reproductive adults ranged from 47 to 72 mm, and the range among lakes was similar among islands (Dewdney, 47.5–69 mm; Lotbiniere, 48–57 mm; Barnard, 53–62 mm; Banks, 59–72 mm) (Fig. S1A). Of the three common lateral plate morphs found in threespine stickleback (low-plated, partially plated, and fully plated), only low-plated morphs (anterior plates, no keel) were found. The mean number of lateral plates ranged from 2 (lateral plate positions 5–6) in several lakes from south Dewdney Island and two lakes from Lotbiniere Island to a high of 6.8 (lateral plate positions 2–8) on Banks Island, the most northerly of

Fig. 2. Representative *Gasterosteus aculeatus* with morphometric variables (abbreviations are defined in Materials and methods). Eight lateral plates are shown at positions LP#1 through LP#8. GAPEW (distance between most posterior positions of left and right premaxillae) is not shown. Inset: ventral view of pelvic girdle.

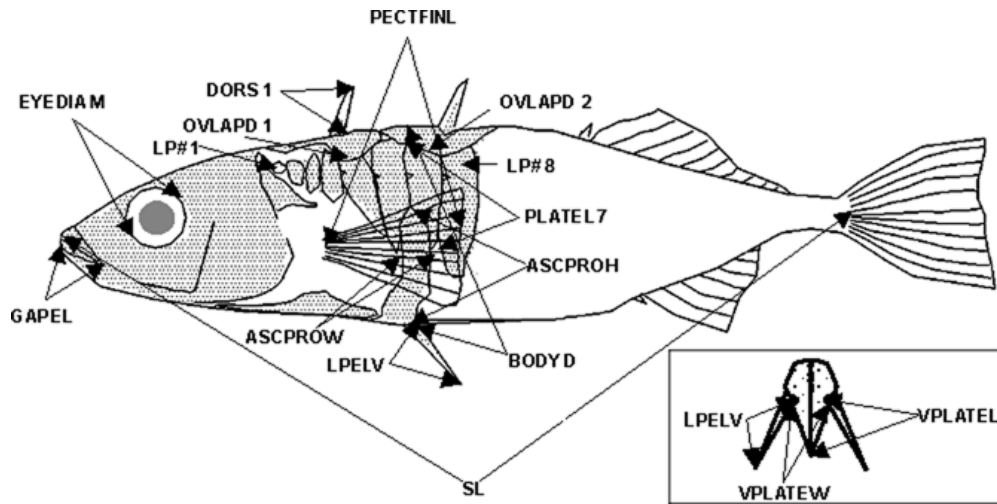


Table 1. Principal component axes from three analyses of morphological variation in 40 freshwater stickleback (*Gasterosteus aculeatus*) populations from the Banks–Estevan archipelago (abbreviations are defined in Materials and methods).

Trait	PC1, all traits	PC2, all traits	PC1, reduced	PC2, reduced	PC1, all fish	PC2, all fish
BODYD	0.47	0.63	0.57	0.58	0.58	0.54
GAPEW	0.00	0.72	0.04	0.73	0.05	0.72
GAPEL	-0.10	0.76	-0.07	0.78	-0.10	0.82
DORS1	0.83	-0.01	0.84	-0.05	0.80	-0.11
LPELV	0.89	-0.07	0.90	-0.09	0.88	-0.12
VPLATEL	0.75	-0.15	0.79	-0.17	0.78	-0.19
VPLATEW	0.60	0.21	0.67	0.17	0.66	0.20
ASCPROH	0.83	0.07	0.86	0.01	0.84	0.03
ASCPROW	0.80	0.06	0.80	0.04	0.77	0.06
PLATEL7	-0.01	0.47	-0.02	0.41	-0.08	0.36
EYEDIAM	-0.02	0.69	-0.08	0.64	-0.14	0.63
PLATENO	0.74	-0.07	0.58	-0.07	0.50	-0.06
RAKERSUP	0.27	-0.13	0.19	-0.16	—	—
RAKERSLO	0.24	-0.29	0.22	-0.30	—	—
OVLAPD1	0.79	0.02	—	—	—	—
OVLAPD2	0.72	-0.11	—	—	—	—
FORKNO	0.52	-0.07	—	—	—	—

Note: See Materials and methods for distinction between “reduced” and “all” analyses. PC1 had large, positive values for traits related to predator defence, such as LPELV, PLATENO, and DORS1. PC2 had large absolute values for trophic traits distinguishing benthic from limnetic stickleback (e.g., GAPEL, BODYD, GAPEW, and RAKERSLO).

the sites (Fig. S1B). Unarmoured stickleback with loss of all plates were infrequent and occurred in only two localities on south Dewdney Island (39 and 41), where 8% and 16%, respectively, of the fish were naked. SL-corrected pelvic spine length was lowest (about 7% SL) on the southern part of the archipelago and was consistently high (about 16% SL) on Banks Island (Fig. S1C). Loss of spines occurred in 21 of the 40 populations and usually at low frequencies (<15%) (Fig. S1D). The exception was one site on Barnard Island (55), where 62% of the fish had spine loss, primarily the first dorsal spine. Trophic morphology also varied. The number of gill rakers ranged from 16.3 on south Dewdney Island (41) to 22.1 on Banks Island (70), but there was considerable variability within each island (Fig. S1E). Size-corrected gape length varied among localities within each island and was lowest on Banks Island (Fig. S1F).

We calculated a cross-correlation matrix of the means for the six traits discussed above (SL, PLATENO, LPELV, DORS1, GAPEL, and total gill rakers (RAKERSUP + RAKERSLO)). Four significant associations emerged (PLATENO and SL-corrected LPELV, $r = +0.71$, $p < 0.001$; PLATENO and total gill rakers, $r = +0.47$, $p = 0.002$; SL-corrected LPELV and total gill rakers, $r = +0.33$, $p = 0.04$; SL-corrected LPELV and SL-corrected GAPEL, $r = -0.33$, $p = 0.04$).

Multivariate population differentiation

Principal components analysis of all size-adjusted metric traits and all meristic traits using the reduced data set yielded two PC axes that accounted for 36% and 18% of the morphological variation. PC1 had large, positive values for LPELV, DORS1, ASCPROH, ASCPROW, PLATENO,

OVLAPD1, and OVLAPD2, indicating stickleback with a well-developed defence apparatus. The all traits PC, which was not analyzed further (see Methods), yielded high loadings on PC1 for OVLAPD1, OVLAPD2, and FORKNO, confirming that PC1 defines defence morphology (Table 1).

PC2 had large, positive values for GAPEL, GAPEW, EYEDIAM, and BODYD and large, negative values for RAKERSUP and RAKERSLO (Table 1). Increased gape and body depth coupled with reduced gill raker number is typical of stickleback adapted to foraging in benthic and littoral habitats. This suggests that increasing canonical coefficients for PC2 are indicative of increasingly benthic morphology. Similar results were obtained when rakers were excluded (i.e., all fish analysis; see Materials and methods), and all subsequent analyses were conducted using the PC axes from the reduced analysis.

There was geographical structuring of the population means for PC1 and PC2 within each of the sexes (Fig. 3). In both males and females, populations from south Dewdney, Lotbiniere, and Barnard islands were characterized by weak development of defence armature (negative PC1), while those from central Dewdney and Banks islands had well-developed defence armour (Figs. 3A, 3B). Trophic structures (PC2) were also geographically variable but showed less concordance between the sexes than did armour (Figs. 3C, 3D).

Nested ANOVA confirmed that morphological variation was partitioned both among islands and among populations within islands (PC1: 36.2% among islands, $F_{[5,34]} = 4.26$, $p < 0.01$; 52.8% among populations nested within islands, $F_{[34,751]} = 95.49$, $p < 0.001$; PC2: 25.1% among islands, $F_{[5,36]} = 7.87$, $p < 0.001$; 14.0% among populations nested within islands, $F_{[34,751]} = 5.56$, $p < 0.001$).

Sexual dimorphism

Sexual dimorphism occurred in univariate means (Tables S1, S2¹). In the majority of populations (>75%), females had larger maximum adult SL and a larger SL-corrected pelvic girdle plate than males did (Wilcoxon signed-ranks test, $p < 0.001$ for both length and width of the plate). In most other comparisons, males had larger SL-corrected traits, including body depth, left pelvic spine length, pectoral fin length, eye diameter, gape length, gape width, gill raker length, mean number of lateral plates, and numbers of dorsal and anal fin rays (Wilcoxon signed-ranks test, $p < 0.002$ in all cases).

Sexual dimorphism indices were calculated for defence (male PC1 – female PC1) and trophic structures (male PC2 – female PC2) (Fig. 4). Males showed slightly greater expression of the defence apparatus relative to females in the majority of localities, and this was accentuated in several populations from Barnard Island. For trophic structures, the dimorphism index was relatively conserved among the majority of lake populations and showed more benthic adaptations in males than in females.

Correlations with biophysical habitat

We tested for associations between ln-transformed morphological traits of stickleback (maximum SL, number of lateral plates, SL-corrected pelvic spine length, SL-corrected gape length, total number of gill rakers) and biophysical at-

tributes of the lake (ln area, ln depth, ln volume, ln calcium, ln conductivity, pH, and percent macrophyte cover). For univariate comparisons, maximum SL was positively correlated only with conductivity ($p < 0.05$). Defence traits (number of lateral plates and SL-corrected pelvic spine length) showed the strongest positive correlations with lake pH ($p < 0.001$). Trophic structures such as gill rakers showed no significant correlations with any lake attributes, while SL-corrected gape length was positively associated with pH ($p < 0.001$).

We then tested whether multivariate morphological differentiation was correlated with our multivariate indices of lake habitat (Table S1¹; Fig. 5). Morphology PC1 was positively correlated with habitat PC2 ($r = 0.52$, $p < 0.01$), indicating that the amount of armour expression was greatest in large lakes with high conductivity. Morphology PC2 was negatively correlated with habitat PC1 ($r = 0.41$, $p < 0.05$), indicating that fish with increasingly limnetic trophic morphology were found in large lakes with low macrophyte cover and that fish with the most benthic trophic morphology were found in small lakes with abundant macrophyte cover.

These associations between multivariate morphology and habitat were replicated, as they occurred within all islands and did not differ among islands (island \times PC1 morphology interaction, $F_{[3,39]} = 0.39$, $p = 0.76$; island \times PC2 morphology interaction, $F_{[3,39]} = 0.86$, $p = 0.47$; test for homogeneity of slopes, ANCOVA; Fig. 5).

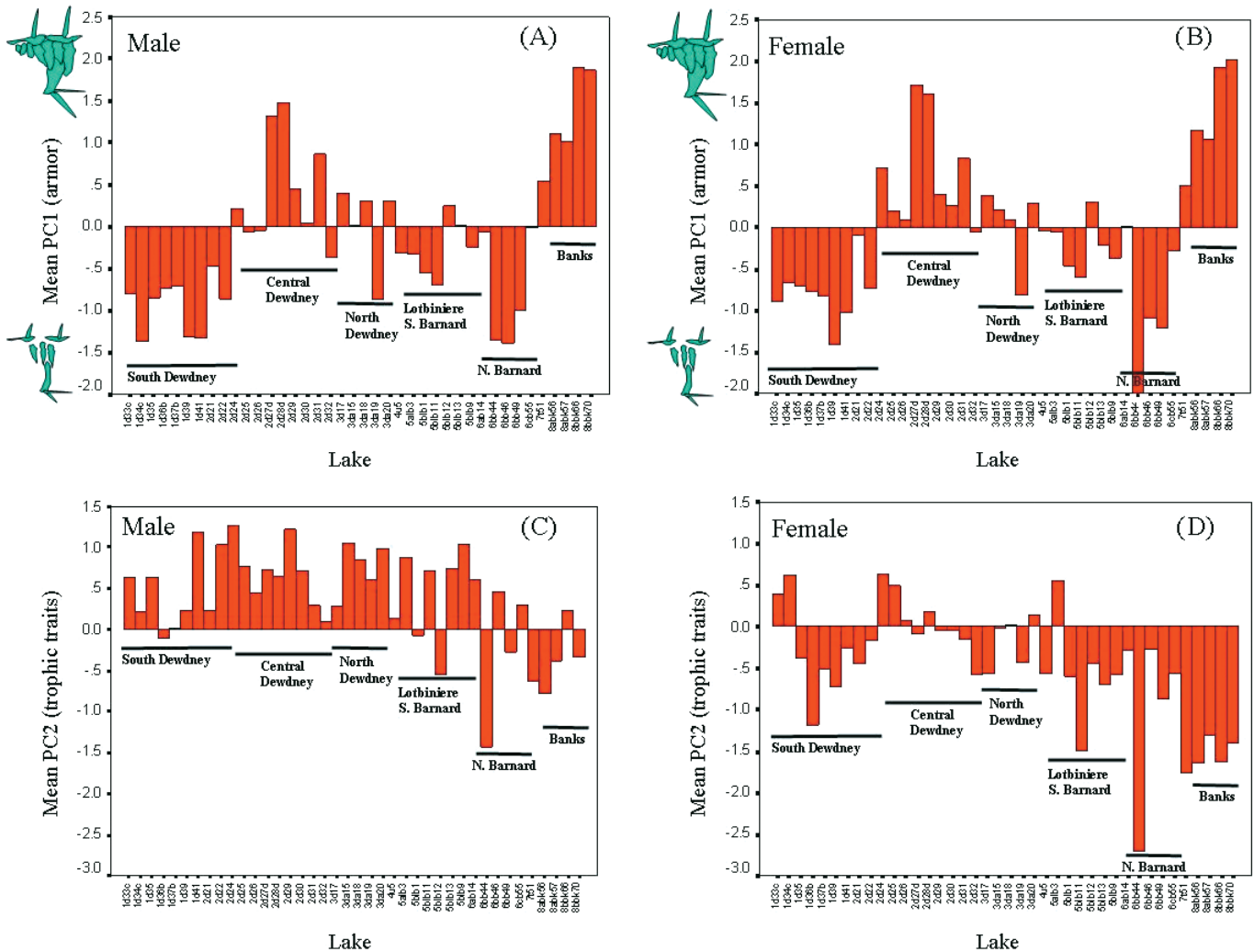
Sexual dimorphism in morphology (male PC1 – female PC1, male PC2 – female PC2) was not correlated with either index of lake habitat ($p > 0.2$ in all cases), although there was a negative correlation with conductivity ($r = -0.35$, $p < 0.03$).

Discussion

The extensive geographical sampling of threespine stickleback in Eurasia and North America over the last century continues to provide evidence for continental, regional, watershed, lake, and microspatial variation in morphology and adaptation, of which the majority is Holocene in origin (for review, see Bell and Foster 1994). Some of the greatest interpopulational variability in stickleback morphology occurs on Haida Gwaii, British Columbia, where large differences in traits such as adult body size and defence armour occur among populations separated by several kilometres, even when there is opportunity for gene flow (Moodie and Reimchen 1976a; Reimchen et al. 1985; Deagle et al. 1996). Much of this variation can be associated with site-specific differences in the selective landscape (Reimchen 1994).

We sampled stickleback from bog ponds and lakes in the Banks–Estevan archipelago to provide a direct comparison with stickleback from the lowland bogs of northeastern Haida Gwaii. We observed substantial univariate and multivariate divergence among the populations, with the directionality of change concordant with that observed on Haida Gwaii for all traits. The most robust defence armature was found in large dystrophic lakes or large lakes with high conductivity. Conversely, the weakest armour expression was found in small ponds with low conductivity, and the greatest extent of spine loss occurred in a higher elevation bog pond (Barnard 55) that was the most geographically isolated from

Fig. 3. Multivariate population differentiation in morphology for 40 freshwater stickleback populations from the Banks–Estevan archipelago, plotted for males and females separately. Values shown are mean principal component scores for PC1 and PC2. High, positive values for PC1 are indicative of well-developed armature (i.e., spines and plates). High, positive values for PC2 are indicative of benthic-like (vs. limnetic-like) morphology (see Table 1 for trait loadings for each axis). (A) Males, PC1; (B) females, PC1; (C) males, PC2; (D) females, PC2. Localities are ordered from south (left) to north (right).



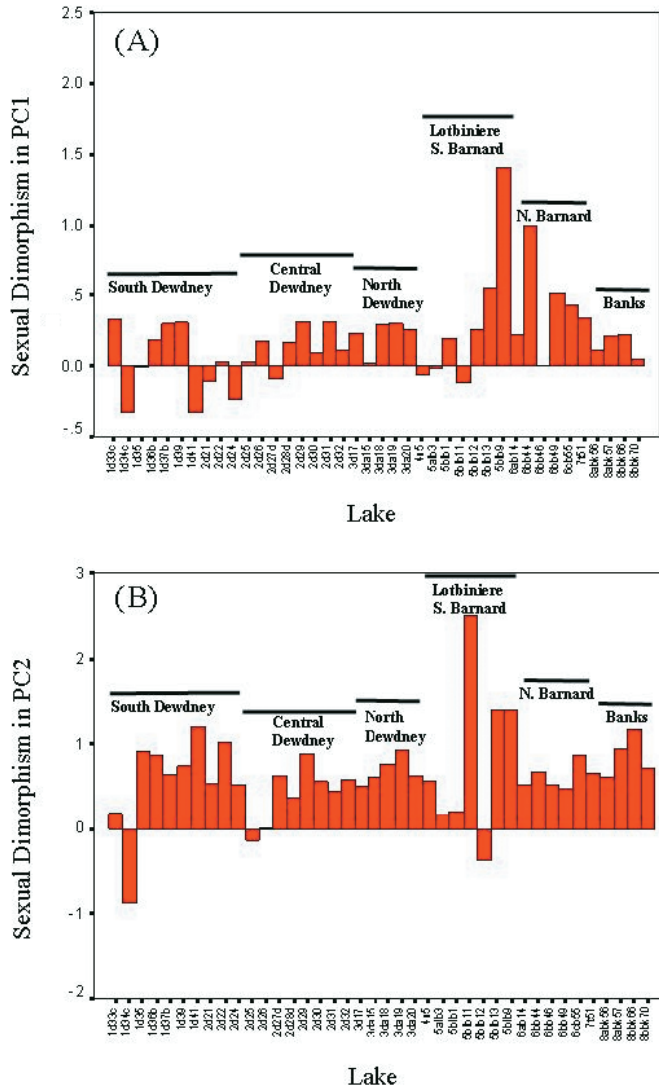
marine waters. Benthic trophic morphology was most prevalent in shallow ponds with extensive macrophytes. These trends are also concordant with studies in Alaska and southern British Columbia (Schluter and McPhail 1992; Bell et al. 1993; Walker 1997). We observed no instances of species pairs like those described in several sites in southern British Columbia (McPhail 1993).

The average adult body length of stickleback observed on the Banks–Estevan archipelago was about 50 mm, similar to that found elsewhere in the circumboreal distribution of the group (for review, see Wootton 1976, 1984). The largest adults on the archipelago, which occur on Banks Island, are 72 mm long, whereas the largest in Alaskan lakes are 79 mm long (Baker et al. 1998) and the largest in bog lakes of nearby Haida Gwaii are 100 mm long (Moodie and Reimchen 1976a). Such populational differences in adult body length probably reflect maximum life span and age structure. Threespine stickleback with a 2- or 3-year maximum life span typically reach sizes of 50 and 70 mm, respectively

(for review, see Wootton 1976); those with a life span of up to 8 years (one of the large-bodied iteroparous populations from Haida Gwaii) may be >80 mm long (Reimchen 1992b). We observed no statistically significant trends between average or maximum body size and any of the biophysical lake variables, although the smallest adult body sizes occurred in the small ponds with extensive littoral macrophytes.

Throughout the geographical distribution of threespine stickleback, the bony defences of these fish exhibit variability among populations (for reviews, see Wootton 1984; Bell and Foster 1994). Marine stickleback represent the ancestral state for freshwater colonization (Bell 1976, 1984) and are usually fully plated with a full dorsal and pelvic spine complement. Freshwater populations with the fully plated condition are common in eastern North America but are much less common in western North America, where a large proportion of the freshwater populations have lost all of the posterior plates and are monomorphic for the low-plated

Fig. 4. Sexual dimorphism in multivariate population differentiation in morphology for 40 freshwater stickleback populations from the Banks–Estevan archipelago. Shown is the difference in mean principal component scores between the sexes (males minus females) for each of the study populations. Thus, values above zero indicate greater values in males and values below zero indicate greater values in females. (A) Sexual dimorphism in PC1; (B) sexual dimorphism in PC2. Localities are ordered from south (left) to north (right).



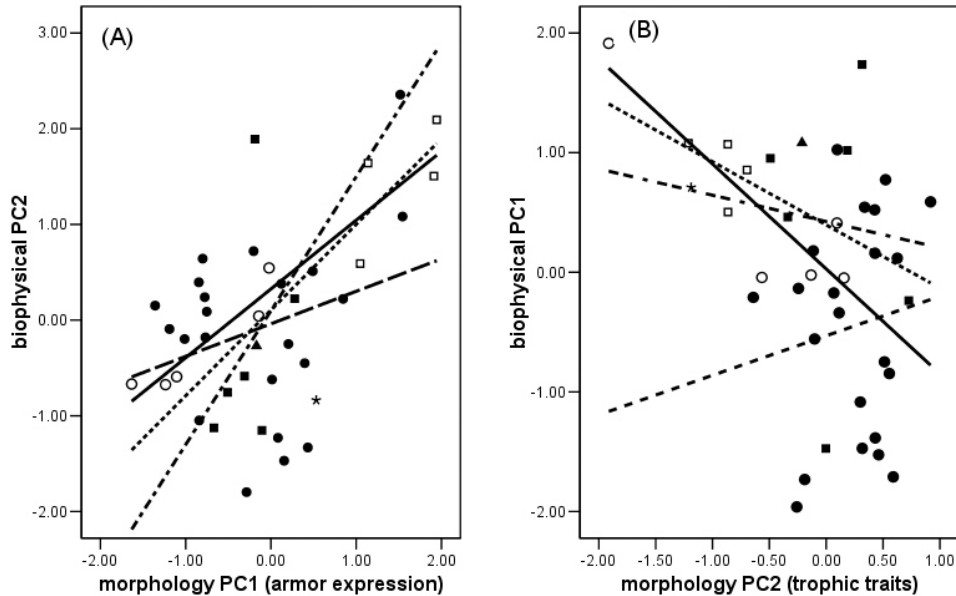
condition (anterior plates and no keel) (Hagen and Moodie 1982). The Banks–Estevan stickleback populations are concordant with this trend. We observed no instances of the partially plated morph, which, when present, can reflect hybridization between the marine and freshwater populations (Hagen 1967; Moodie 1972) as well as selective differences among populations (Baumgartner and Bell 1984).

Multiple mechanisms for the major loss of posterior plates in freshwater stickleback populations have been proposed. In early investigations, plate reduction was thought to represent an environmentally induced phenotypic response to a combination of low salinity and warm temperatures, but subsequent evidence showed that loss of posterior plates was heritable and controlled by several major loci (Hagen 1973;

for review, see Wootton 1984). Hagen and Gilbertson (1972) found no relationship between lateral plate characteristics and water chemistry (e.g., pH, conductivity, and total dissolved solids), although more recent evidence indicates that reduced ion composition could be a major selective agent promoting plate loss (Giles 1983; Bourgeois et al. 1994). We found no correlation between plate reduction and lake calcium levels, but we did observe that populations with weak defence structures were found in lakes with low conductivity and low pH, both of which provide a broad proxy for low calcium concentration (Bierhuizen and Prepas 1985; Kalff 2002; Jones et al. 2003). However, despite this association, we are unable to exclude an alternative mechanism related to predation regime. Smaller lake size and lower conductivity are also associated with basic shifts in the assemblage of predators. Salmonids are uncommon in low-pH lakes (Reimchen 1992a), which increases the importance of avian piscivores. This shift from toothed to compression predators can allow alternative defences to evolve, including loss of posterior plates for increased trunk flexibility and increased burst acceleration during predator pursuit (Reimchen 1992c, 2000; Bergstrom 2002). As well, in the smallest lakes, macroinvertebrates might generate selection favouring armour reduction, and these predators are most prevalent in the absence of predatory fish (Reimchen 1980, 1994; Reist 1980; Reimchen and Nosil 2002, 2004; Vamosi 2002).

Our multivariate index of the defence apparatus in stickleback showed considerable geographical structuring within and among islands, including weak expression throughout South Dewdney and North Barnard islands and robust expression in several localities from central Dewdney Island and all localities from Banks Island. Multiple studies have suggested that stickleback populations exposed to predatory vertebrates (birds and fish) have increased expression of morphological defences (e.g., six or seven lateral plates) relative to those not exposed to predatory vertebrates (Hagen and Gilbertson 1972; Moodie et al. 1973; Moodie and Reimchen 1976a; Gross 1978; Reimchen 1983). Avian piscivores were common throughout the Banks–Estevan archipelago, and all ponds and lakes would be accessible for foraging. During our survey period in June, predatory fish were not observed in the majority of lakes other than those on Banks Island and are probably less important than avian piscivores, at least during summer months. However, the presence of juvenile coho salmon and cottids in many streams connecting the ponds and lakes to marine waters indicates opportunity for access to the ponds and lakes, particularly in autumn and winter when lake levels and stream discharge rates are much greater than they are in summer (Reimchen 1994). Differences in the relative contributions of these itinerants among localities and seasons constitute an important difference in the selective landscape among stickleback populations of Haida Gwaii (Reimchen 1980, 1994; Reimchen and Nosil 2002, 2004) and may similarly contribute to the differences among Banks–Estevan stickleback populations. The majority of the Banks–Estevan populations had a mean plate count between 4 and 5, indicative of an avian-dominated predator landscape and a selective regime parallel to that observed in the *Sphagnum* lowlands of Haida Gwaii (Reimchen 1994).

Fig. 5. Relationships between indices of multivariate biophysical habitat and indices of multivariate morphology for 40 freshwater stickleback populations from the Banks–Estevan archipelago. Symbols denote the island a population originates from and separate regression lines are shown for islands with multiple populations: \blacktriangle , Uni; $*$, Trutch; \blacksquare (alternating dashed line), Lotbiniere; \bullet (line of long dashes), Dewdney; \square (line of short dashes), Banks; \circ (solid line), Barnard. The relationships between biophysical habitat and morphology did not differ among islands (both $p > 0.25$, test for homogeneity of slopes). (A) PC1 for morphology (armour expression) is positively related to PC2 for habitat. (B) PC2 for morphology (trophic structures) is negatively related to PC1 for habitat.



Evolution of means and variances

Previous work on Banks–Estevan stickleback populations (Nosil and Reimchen 2005) examined levels of phenotypic variance within populations in relation to ecological opportunity. The results indicated that variance in two armour traits (plate number and dorsal spine length) decreased with lake size, whereas variance in two trophic traits (gape width and pectoral fin length) increased with lake size. Reduced vertebrate predation in small bog lakes may allow increased variance in defensive structures without costs to fitness. Conversely, larger lakes with well-developed littoral and limnetic zones will have greater trophic niche diversity than smaller lakes with only littoral zones. The present study provides additional insight into the evolution of morphological diversity within and among these populations. Both trait means and trait variances were affected by biophysical lake characteristics (although the latter was affected only by lake size). However, trait means showed a great deal of geographic structure, whereas trait variances did not. Thus, trait means and variances were not strongly correlated.

Two factors might account for differences in the evolution of means versus variances. First, trait means might be more strongly affected by colonization history, with populations evolving variability according to ecological opportunity but mean phenotype also being affected by the mean of the ancestral colonizing population. Second, the geographic structuring of means suggests that regional habitat characteristics result in selective landscapes that affect the evolution of trait means across relatively broad geographic areas (i.e., across large portions of islands). Conversely, variances might be free to evolve primarily as a function of lake size and somewhat independently of these larger scale processes.

Sexual dimorphism

The expression of sexually dimorphic traits in threespine stickleback has received considerable attention with respect to the divergent reproductive life histories of the sexes (Wootton 1984), yet the importance of ecological differences between the sexes has received less interest. In several lakes of Haida Gwaii, males tend to be more littoral and more benthic than females (Reimchen and Nelson 1987), have slightly more lateral plates than females do in lakes with predatory salmonids (Moodie 1972; Moodie and Reimchen 1976a), and have shorter spines and less armour than females do when predatory salmonids are absent (Reimchen 1980; Reimchen and Nosil 2004). The sexes also exhibit persistent differences in relative infection rates by four parasitic species (Reimchen and Nosil 2001). Threespine stickleback exhibit male–female differences in jaw and tooth structures (Caldecutt and Adams 1998; Caldecutt et al. 2001). In stickleback from the Banks–Estevan archipelago, we observed concordant shifts in morphology between the sexes within and among islands, yet the sexual dimorphism showed remarkable consistency. Males had slightly greater expression of defence armour, including proportionately longer spines and more lateral plates, and had greater expression of benthic adaptations than females did. As there are regular spatial differences in the use of littoral and limnetic habitats by avian and piscine predators (Reimchen 1994), such sexual dimorphism in armour can be expected. Avian piscivores that pursue fish under water tend to be more prevalent in open regions of the lake, whereas predatory trout such as *O. clarki* are more prevalent near shore; therefore, on average, females should be subject to increased avian predation and males should be subject to increased trout predation. If this is true, one predicts that the extent of

sexual dimorphism should vary with lake biophysical characteristics. Although we found variability in sexual dimorphism among localities, we observed no predictable relationships between the multivariate indices of either armour dimorphism or trophic dimorphism and multivariate lake descriptors. Differences between the sexes detected in this study reflect trends similar to the ecological differentiation of the benthic and limnetic species pairs seen in several lakes from southern British Columbia (Baumgartner et al. 1988; Schluter and McPhail 1992; Rundle et al. 2000; Schluter 2000; Vamosi and Schluter 2004). Thus, the processes structuring the evolution of sexual dimorphism and speciation may be similar (Bolnick and Doebeli 2003; Bolnick 2004), such that ecological processes play a central role in structuring diversity within populations and divergence between them.

Relict forms

One rationale for this investigation of the Banks–Estevan archipelago was to test for the extent of divergence of stickleback among a range of bog habitats comparable to those from the northeastern edge of Haida Gwaii, where the most divergent unarmoured stickleback have been identified (Moodie and Reimchen 1976a; Reimchen 1992a; Deagle et al. 1996). Although small bog ponds are widespread on the Banks–Estevan archipelago, we found very few instances of major armour reduction. We observed naked fish (no plates) in only two localities and at low frequencies. Loss of pelvic spines was not observed in any sample, yet this occurs in at least four sites on Haida Gwaii (Reimchen 1992a) and occasionally in southern British Columbia (McPhail 1993) and Alaska (Bell and Ortí 1994). One possibility is that such unarmoured populations have evolved but have not persisted over extended periods. Seasonal ice cover of the ponds is much more extensive on the Banks–Estevan archipelago than on Haida Gwaii, owing to lower winter temperatures near the continent, and this would result in a higher likelihood of extinction during prolonged periods of freezing. Local ecotypes restricted to small ponds could be lost under such conditions. Additionally, higher winter extinction rates could occur in bog lakes on the Banks–Estevan archipelago, as these were much less acidic than those on Haida Gwaii, and this higher pH would facilitate decomposition and oxygen depletion, as has been documented for nonhumic lakes in the eastern United States (Tuchman 1993).

The greatest incidence of armour reduction in the Banks–Estevan sites occurred at Barnard 55, where 60% of the fish lacked the second dorsal spine. This locality was exceptional in that it was the most isolated and had the highest elevation among the surveyed lakes. The high gradient of the stream between the lake and the ocean might restrict access by anadromous salmonids; on Haida Gwaii, such restriction produces a selective landscape dominated by avian piscivores and macroinvertebrates, which can lead to spine loss and polymorphism (Reimchen 1980; Reimchen and Nosil 2002).

The most distinctive population observed in the present study occurred in a single locality on Banks Island (Banks 70). Stickleback in this lake are the most melanistic and most robustly armoured (highest PC1 values, highest plate counts), have the largest body size, and exhibit a very streamlined body with a sharply tapered head. These stickle-

backs were strikingly similar in shape and appearance to the endemic giant black stickleback from Drizzle Lake and Mayer Lake on the eastern side of Graham Island, 140 km to the northwest (Reimchen et al. 1985). Such similarity in morphology among disjunct populations can be due to a common selective landscape or common ancestry. Based on investigations of the giant stickleback from Haida Gwaii, this selective landscape is currently thought to be shaped by a diverse assemblage of salmonid and avian predators occurring in large (>100 ha) dystrophic lakes with low calcium levels (<1 ppm), low pH (5.5), and heavily tannin-stained waters (<70% transmission at 400 nm) (Moodie 1972; Reimchen and Douglas 1984; Reimchen 1989, 1991, 1994). Predation regime could not be evaluated during the short survey on Banks Island, but this island's proximity to marine waters (500 m) and its low elevation (ca. 15 m) suggest the potential for full access by anadromous fish and suitability for avian piscivores. Our limnological data, however, suggest that Banks 70 has only limited similarity to the Haida Gwaii lakes with giant stickleback, as the lake is small (9 ha) and has higher calcium levels (7 ppm), a neutral pH (6.8), and high water clarity (>90% transmission at 400 nm), which is typical of many lakes on the Banks–Estevan archipelago, none of which have stickleback resembling those from Banks 70. An alternative interpretation of the similarity in body form between stickleback in Banks 70 and those on Haida Gwaii is common ancestry. Recent colonization or gene flow seems improbable given the 140 km of open marine waters separating the freshwater lakes of the two archipelagos. However, there is expanding palynological and molecular phylogenetic evidence that the continental shelf separating the two archipelagos was a potentially ice-free terrestrial refugium during the last glacial advance (Fladmark 1979; Warner et al. 1982; Byun et al. 1997, 1999; Reimchen and Byun 2005). The presence of large freshwater lakes on this refugium (Josenhans et al. 1995) opens the possibility that relict giant stickleback from the early Holocene colonized both Haida Gwaii and one or more lakes on Banks Island. Future molecular data will allow the possibility of common ancestry to be evaluated.

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