Origins of endemic stickleback from the Queen Charlotte Islands: mitochondrial and morphological evidence

B.E. Deagle, T.E. Reimchen,[1] and D.B. Levin

Abstract: Recently, two divergent mitochondrial lineages were described in a survey of 12 scattered populations of the threespine stickleback (*Gasterosteus aculeatus*) on the Queen Charlotte Islands (QCI), western Canada, one of which, possibly a relict lineage, was subsequently shown to characterize stickleback from the western Pacific near Japan. In the present study, we assayed 985 fish from 85 QCI populations for mitochondrial lineage using a restriction enzyme test. Our data indicate that the relict lineage was largely limited (18 of 20 populations) to adjacent watersheds in the northeast corner of the QCI close to a suspected glacial refugium, but we also found the lineage in two remote lakes on the west coast of the QCI, distant from any known refugia. We obtained a sample of 33 stickleback from the mid-Pacific and found both mitochondrial DNA (mtDNA) lineages, strongly suggesting ongoing dispersal of these fish across the Pacific, and inconsistent with previous suggestions of relict status for one of the lineages. Morphologically derived traits, such as loss of armour and loss of spines, occurred in both lineages, but these were more prevalent in the Japanese lineage. Surprisingly, 19 localities had both lineages, and within each of these there were no significant univariate or multivariate associations between lineages and morphology, suggesting few if any reproductive barriers between the divergent mtDNA lineages.

Résumé : Récemment, deux lignées mitochondriales divergentes ont été décrites à partir d'un inventaire de 12 populations dispersées de l'Épinoche à trois épines (*Gasterosteus aculeatus*) dans les îles de la Reine-Charlotte, dans l'Ouest canadien, lignées dont l'une, peut-être relicte, possède des caractéristiques semblables à celles des épinoches de l'ouest du Pacifique, près du Japon. Nous avons utilisé des enzymes de restriction pour faire l'analyse de l'ADNmt de 985 poissons appartenant à 85 populations de l'archipel. Nos données indiquent que la lignée relicte est en grande partie restreinte (18 de 20 populations) à des bassins adjacents du coin nord-est de l'archipel, aux environs de ce que l'on soupçonne être un refuge glaciaire, mais la lignée à également été trouvée dans deux lacs retirés de la côte ouest de l'archipel, loin de tout refuge connu. Nous avons analysé l'ADN mitochondrial (ADNmt) de 33 épinoches du milieu du Pacifique et y avons constaté la présence des deux lignées, ce qui indique que ces poissons se dispersent encore actuellement dans tout le Pacifique, et qui contredit les hypothèses antérieures d'une lignée relicte. Des caractéristiques morphologiques évoluées, telles la perte de la cuirasse et la perte d'épines, ont été observées chez les deux lignées, mais plus fréquemment chez les épinoches de la lignée japonaise. Étonnamment, les deux lignées ont été retrouvées ensemble en 19 localités et, à aucune d'elles nous n'avons constaté d'association unidimensionnelle ou multidimensionnelle entre lignée et morphologie, ce qui indique qu'il existe peu de barrières génétiques entre les deux lignées divergentes d'ADNmt. [Traduit par la Rédaction]

Introduction

The threespine stickleback (*Gasterosteus aculeatus*), widely distributed in marine and coastal fresh waters of the northern hemisphere, has become an important taxon for evaluating a diverse range of evolutionary processes. Freshwater colonization by morphologically conservative anadromous stickleback has resulted in a radiation of form and behaviour exceptional among fishes (reviews in Wootton 1976, 1984; Bell and Foster 1994). On the Queen Charlotte Islands (QCI) off the west coast of Canada, stickleback display morphological variation over short distances as great as that seen throughout the circumboreal distribution of the species (Moodie and Reimchen 1973, 1976*a*; Reimchen et al. 1985). Natural selection is a major factor in the differentiation seen in these insular populations (Moodie 1972; Moodie and Reimchen 1976*b*; Reimchen 1980, 1983, 1994). Uncertainties remain as to whether the more divergent forms have been recently derived in postglacial periods or are relicts of an ancient lineage that may have survived in ice-free refugia postulated for the area (Foster 1965; Kavanaugh 1980; Warner et al. 1982; Schofield 1989; Ogilvie 1989; Taylor 1989).

Preliminary comparisons of restriction fragment length polymorphisms for mitochondrial DNA (mtDNA) of the putative marine ancestor and several divergent freshwater stickleback populations, including the giant stickleback and a spine-deficient stickleback, indicated recent differentiation (Gach and Reimchen 1989). Further mitochondrial studies of 12 QCI stickleback populations (O'Reilly et al. 1993) identified two major lineages differing by 2.4% sequence divergence; one of the lineages characterized marine and

Received April 24, 1995. Accepted January 5, 1996.

B.E. Deagle, T.E. Reimchen, and D.B. Levin. Department of Biology, University of Victoria, Box 1700, Victoria, BC V8W 2Y2, Canada.

[1] Author to whom all correspondence should be addressed (e-mail: reim@uvvm.uvic.ca).

the majority of freshwater populations and the second lineage was limited to four populations exhibiting a highly derived unarmoured morphology. This second lineage was found only in populations from the northeast corner of the islands near a postulated Wisconsin refugium. Stickleback in one of these populations carried a highly anomalous parasitic dinoflagellate (Reimchen and Buckland-Nicks 1990). These data were most consistent with an extended preglacial history for these unarmoured stickleback (O'Reilly et al. 1993).

A subsequent global survey of stickleback mtDNA cytochrome *b* sequences identified a large genetic break between populations from the western Pacific area (Japanese lineage) and from most populations in Europe and North America (Euro-North American lineage) (Ortí et al. 1994). Samples from the QCI were unusual because they included representatives from both lineages. The relict lineage identified by O'Reilly et al. (1993) in the unarmoured stickleback corresponded to the Japanese cytochrome *b* lineage, while the second, more common QCI lineage corresponded to the Euro-North American cytochrome *b* lineage. The Japanese lineage was also found in several localities near putative refugia in Alaska. Ortí et al. (1994) concluded that these disjunct populations within the Japanese lineage represent relicts that survived in ice-free areas of coastal North America.

Despite the apparent divergent histories of the two lineages, three of the QCI populations that had highly derived morphologies contained both lineages (O'Reilly et al. 1993). Intrapopulation mtDNA polymorphisms of this type have been observed previously in natural populations (Moran and Kornfield 1993), but the apparent restriction of this polymorphism to small, morphologically derived populations makes this case peculiar. Lineage sorting in small populations typically leads to haplotype fixation (Avise et al. 1987). Reproductive isolation between the lineages could account for the persistence of both lineages, and if so, one suspects that there will be ecological differentiation between the lineages which should be reflected in some morphological traits.

Our objectives in this study are, firstly, to determine the broad geographical distribution of the "relict" lineage to further focus the search for the putative refugium, and secondly, to test for any associations between mtDNA lineage and morphology within and among localities. This information will provide a historical framework for evaluating the remarkable morphological radiation among the insular populations.

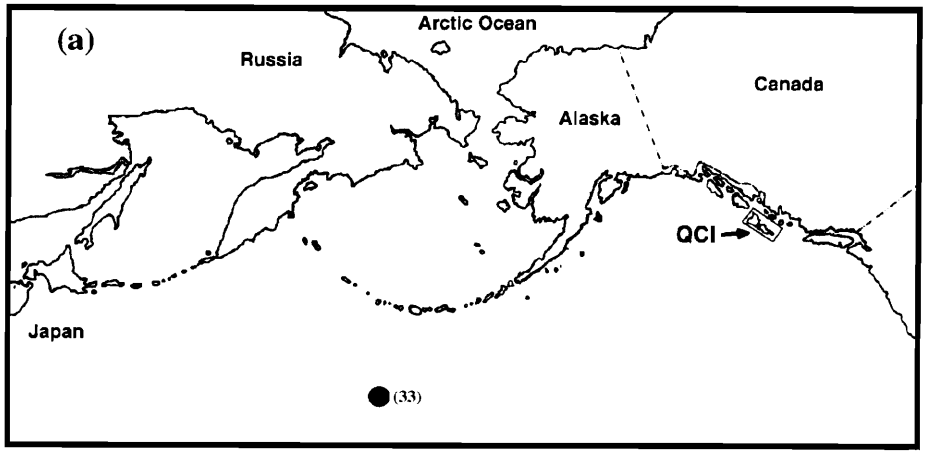
Materials and methods

In total, 974 stickleback from 82 populations were collected throughout the QCI in the summers of 1993 and 1994 (Figs. 1 and 2). Sample sites included lakes and ponds and to a lesser extent streams and estuaries. Most sites were in the northeast part of Graham Island, which is where the greatest number of stickleback populations occur, including most of the divergent forms. Fish were captured using standard minnow traps. An average of 12 specimens per locality were kept and stored individually in 95% ethanol until further processing. Toward the completion of the study, specimens of threespine stickleback were collected by K. Myers and K. Negasawa from the mid-Pacific Ocean (45°31'N, 179°24'W). These were obtained from the stomachs of steelhead and pink salmon caught on July 24, 1994, by a Japanese research vessel. These stickleback were fully intact and only weakly digested, suggesting that they had been swallowed close to the area where the salmon were caught.

Total cellular DNA was isolated from 0.1–0.5 g of muscle tissue. The tissue was ground in hexadecyltrimethylammonium bromide (CTAB) buffer (100 mM Tris–HCl, pH 8.0; 1.4 M $NaCl_2$; 20 mM EDTA; 2% CTAB; 0.2% 2-mercaptoethanol) at 60°C and incubated in the same buffer for 30 min at 60°C. The DNA was purified with chloroform – isoamyl alcohol and precipitated with ethanol – ammonium acetate (Doyle and Doyle 1987). To distinguish between the two divergent lineages a restriction-enzyme test was used. Although this method does not detect variation within each of the lineages, it allows the examination of a large number of individuals, which is critical for accurate mapping of the distribution of the two groups. The sequences of a 747 base pair (bp) fragment of the cytochrome *b* gene from the Japanese and Euro-North American lineages are known (Ortí et al. 1994). These sequences were analyzed using Mapdraw (DNASTAR Inc., Madison, Wis.) for restriction-site differences. A *Nsi*I site is present at position 288 of the Euro-North American lineage but is absent from the Japanese lineage. *Nla*III cuts both lineages at position 399 and has additional diagnostic restriction sites on the Euro-North American lineage fragment. Polymerase chain reaction (PCR) was used to amplify a 831-bp fragment containing the diagnostic restriction sites. Primers used were L14724 (5'-CGAAGCTTGATATGAAAAACCATCGTTG-3') and H15525 (5'-TTTGCAGGGGTAAAATTATCAGGAT-3') (Ortí et al. 1994). Amplifications were carried out in a total volume of 20 μL containing 1 μM of each primer, 100 mM Tris (pH 8.8), 0.01% Triton X-100, 2.5 mM $MgCl_2$, 10–1000 ng genomic DNA, and 0.5 units of Taq Polymerase (Cetus). Resulting PCR products were digested without purification with *Nsi*I and fragments were separated by electrophoresis through a 2.0% agarose gel. If the PCR product cut into two fragments (337 and 494 bp) the individual was assigned to the Euro-North American lineage. If the PCR product remained uncut, it was digested with *Nla*III to verify that the individual belonged to the Japanese lineage. The mtDNA from some individuals could not be amplified using the above primers, so the L14724 primer was used with H15149 (5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3') (Kocher et al. 1989), yielding a PCR product 485 bp long. This PCR product contained the diagnostic *Nsi*I site. A HincII site present only in the Japanese lineage at position 228 of the known sequence was also used to assay some individuals after this PCR product was obtained.

Morphological data were obtained from all the QCI stickleback collected ($N = 974$), in addition to 80 stickleback from five localities sampled by O'Reilly et al. (1993). Each fish was measured for standard length (SL), maximum body depth, and pelvic spine length, and the number of lateral plates on the left side of the body, position of the lateral plates, presence–absence of spines (first, second, and third dorsal, left pelvic, right pelvic, and anal), and sex were determined. Reductions in number of spines were grouped into two categories: (1) all spines present and (2) one or more spines absent. Body depth and pelvic spine length, which are body-size dependent, were adjusted to an average body length of 50 mm SL, based on the total sample regression slope for each trait (Hagen and Gilbertson 1972). Although slopes differed visually among populations, sample sizes were too low to detect statistical departures from equality and we have assumed equality of slopes. As an estimate of average adult body size for the populations, we used data from larger samples collected during previous surveys of these lakes (Reimchen et al. 1985; Reimchen 1992*a*; T.E. Reimchen, unpublished data). Stream distance (km) from the sampling locality to the ocean was measured on 1 : 50 000 ordinate survey maps and log-transformed. Stream channels on these maps are frequently incomplete for small lakes and the distances are only approximations. We examined the association between DNA lineage and morphology using (*i*) a correlation matrix for the total data set based on mean population values for each variable, (*ii*) multiple regressions for the total data set, and for a reduced data set, including only localities with both lineages, and (*iii*) Wilcoxon's signed-rank tests for individual localities with both lineages. All statistical tests were run with SPSS (release 6.0; Norusis 1993). For assessing

Fig. 1. (*a*) Location of the Queen Charlotte Islands (QCI) and the collection site in the open Pacific (●). (*b*) Locations of study populations of threespine stickleback on the Queen Charlotte Islands (excluding the northeast corner of Graham Island; see Fig. 2). Populations marked with an asterisk include samples from O'Reilly et al. (1993). The sample size is 12 unless otherwise noted in parentheses.



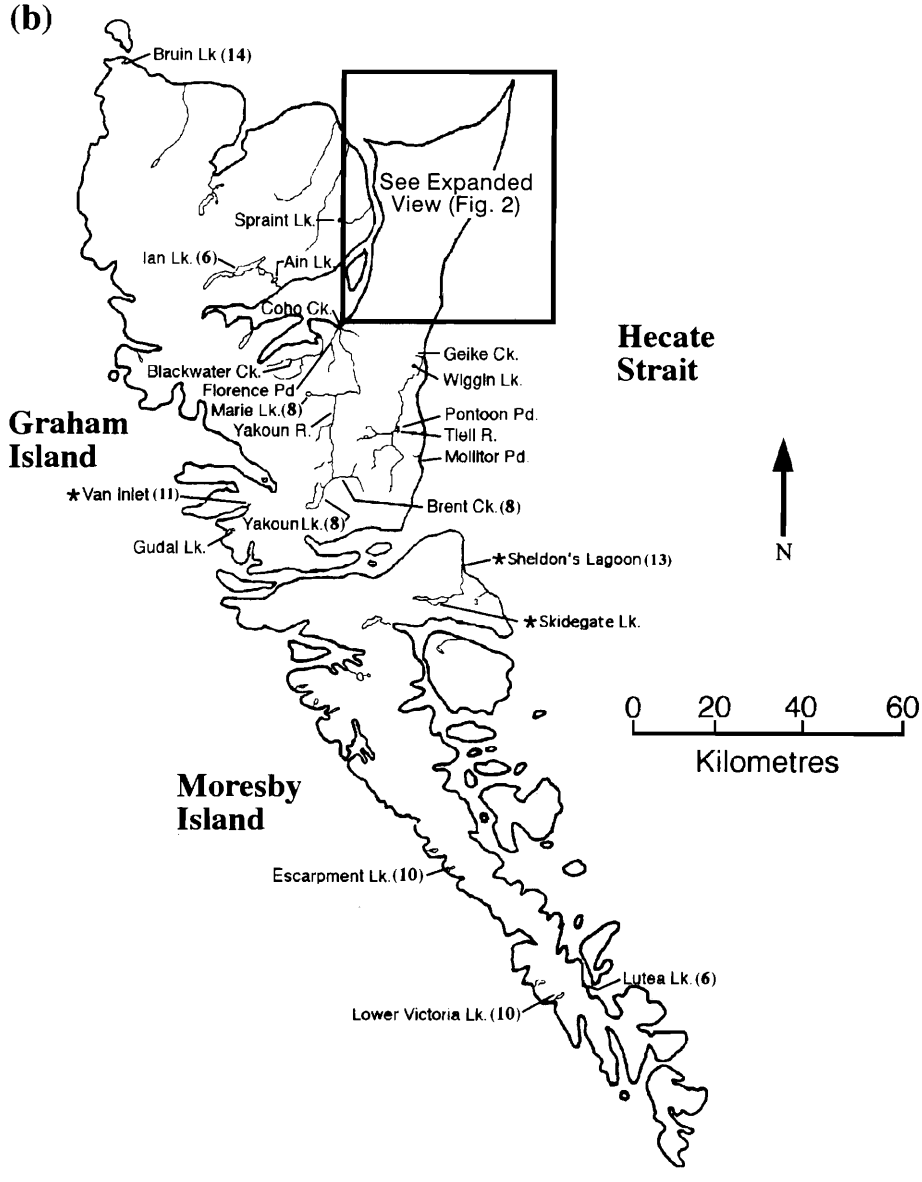
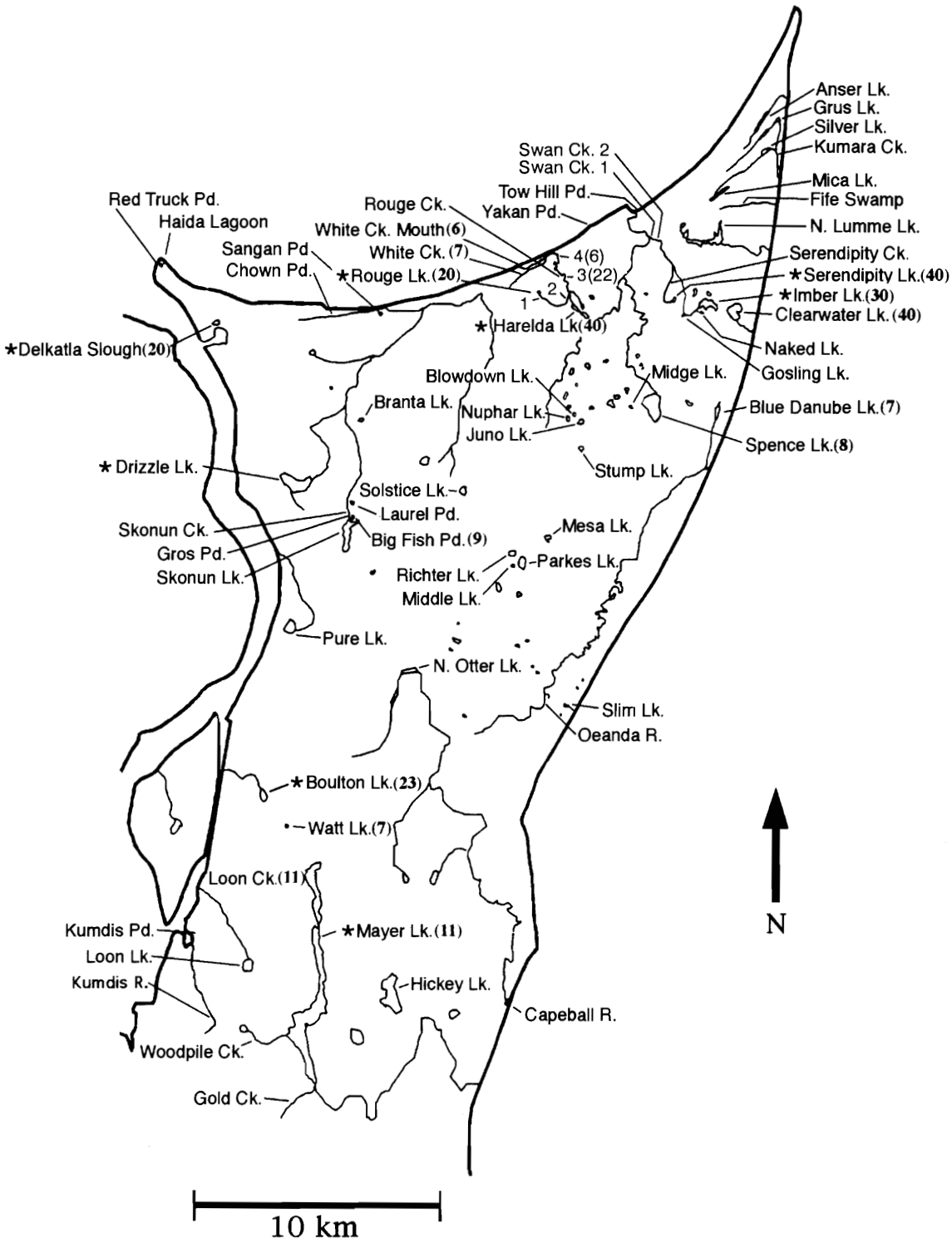


Fig. 2. Location of study populations of threespine stickleback from the northeast corner of Graham Island. Populations marked with an asterisk include samples from O'Reilly et al. (1993). The sample size is 12 unless otherwise noted in parentheses.

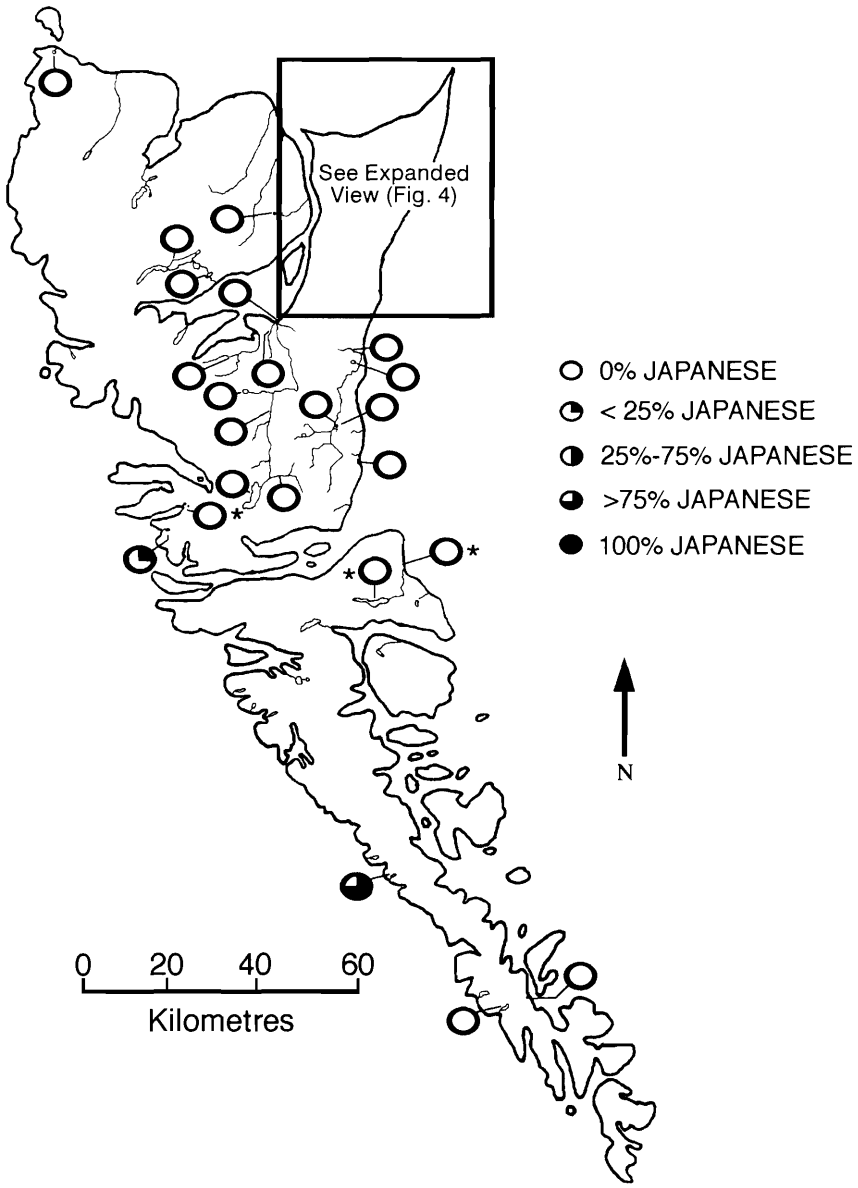


the significance of multiple comparisons in the correlation matrix and the signed-rank tests, we calculated table-wide probabilities using the sequential Bonferroni test (Rice 1989).

Results

The Japanese lineage was identified from 20 QCI population samples, including the 4 populations in which the lineage had been found previously (O'Reilly et al. 1993). Only 1 population was monomorphic for the Japanese lineage (N = 20), while 19 populations contained fish from both of the lineages. Samples from the remaining 62 populations included in this study consisted entirely of fish from the Euro-North American lineage (Figs. 3, 4). The population samples containing the Japanese lineage were mainly from the north-

Fig. 3. Geographic distribution of the two mtDNA lineages on the Queen Charlotte Islands (excluding the northeast corner of Graham Island; see Fig. 4). An asterisk indicates that samples from O'Reilly et al. (1993) are included.



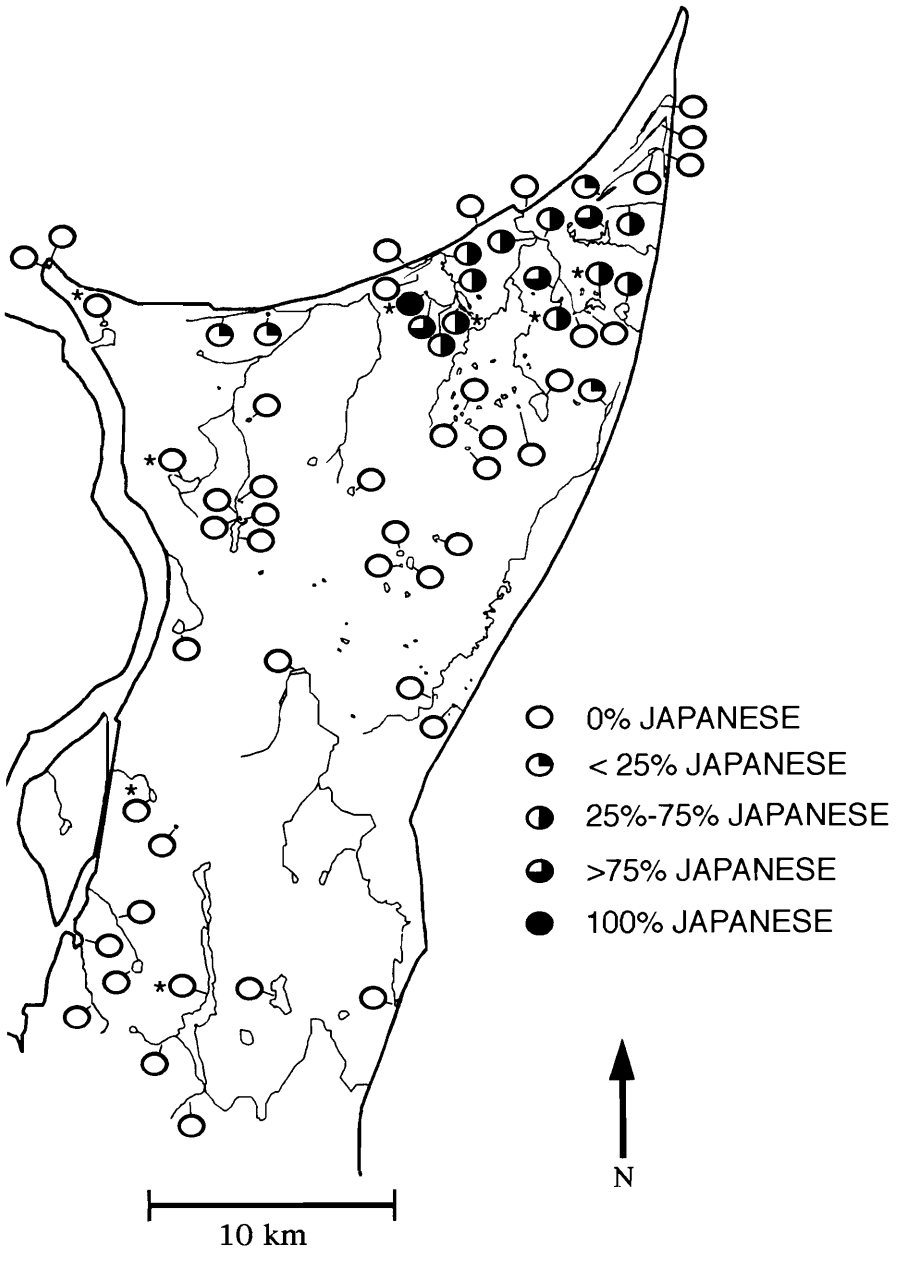
east corner of Graham Island. There were two exceptions, Escarpment Lake and Gudal Lake, which are both on the west coast of the QCI. All QCI population samples included in this study that contained fish with the fully plated morphology, which is ubiquitous among marine and recently derived freshwater isolates of the species, were found to belong to the Euro-North American lineage. A single fully plated fish from an estuary at the north of Graham Island (Delkatla Slough) lacked the diagnostic *Nsi*I restriction enzyme site, but *Nla*III and *Hinc*II restriction fragments showed that the fish belonged to the Euro-North American lineage. Among the sample of 33 stickleback from the site in the mid-Pacific, both mtDNA lineages were identified: 27 individuals belonged to the Japanese lineage and 6 to the Euro-North American lineage.

Previous studies showed that the fish from Rouge Lake were characterized by reduction in relative spine length, frequent reduction in number of spines (73% of individuals), and frequent loss of all lateral plates (62% of all individuals, $\bar{x} = 0.6$) (Reimchen 1984), and this was the only sample ($N = 10$) monomorphic for the Japanese lineage (O'Reilly et al. 1993). Consequently, we suspected that these morphological traits might characterize QCI fish within the Japanese lineage.

A cross-correlation matrix of DNA lineage (percentage with Japanese lineage) against stream distance and mean values of morphological parameters is shown in Table 1. The incidence of the Japanese lineage was not statistically correlated with stream distance, SL, relative body depth, or number of lateral plates, but it was significantly and inversely correlated with relative spine length and directly correlated with incidence of spine loss, both of which were highly correlated with each other.

Multiple-regression analysis using the population means of the seven variables explained only a small proportion of the variability between the lineages ($R^2 = 16\%$, ANOVA,

Fig. 4. Geographic distribution of the two mtDNA lineages at the northeast corner of Graham Island. An asterisk indicates that samples from O'Reilly et al. (1993) are included.



$P < 0.001$). Examination of the standardized regression (β) coefficients shows that incidence of spine loss (SPLOSS) was the only variable that contributed significantly to the regression equation (Table 2). Although an important predictor of lineage, SPLOSS is not restricted to populations with the Japanese lineage (Fig. 5). In Boulton Lake, about 80% of the stickleback have lost spines, yet the Japanese lineage has not been detected in this population.

The statistical association between SPLOSS and incidence of the Japanese lineage in the total data set might be a geographical and statistical artifact. Spine loss among these populations occurs only in the muskeg lowlands of northeast Graham Island, possibly as a result of functional adaptations (Reimchen 1980, 1992*a*), and this could be coincidental to the prevalence of the Japanese lineage in the area. Furthermore, many of the 67 samples lacking the Japanese lineage occur in more mountainous habitats, where fish are exposed to a predation regime that favours completely spined phenotypes (Reimchen 1994). To obtain a better evaluation of DNA lineage and morphology, we excluded all localities that were monomorphic and performed the multiple regression on dimorphic samples. Using the same variables as with the total data set, these data (Table 2, mixed populations.) yield a stronger overall association (multiple $R^2 = 56\%$, $F = 21.6$, $P < 0.001$), but among the seven variables, relative body depth (BD50) was the only significant variable in the regression, and it was inversely related to frequency of the Japanese lineage. Spine loss (SPLOSS) and relative spine length (SP50), the two significant variables in the correlation matrix, did not account for any significant component of the

Fig. 5. Scatterplot of the frequency of the Japanese mtDNA lineage (DNAJP) against spine loss among samples. The star represents 63 localities (names are excluded to minimize clutter).

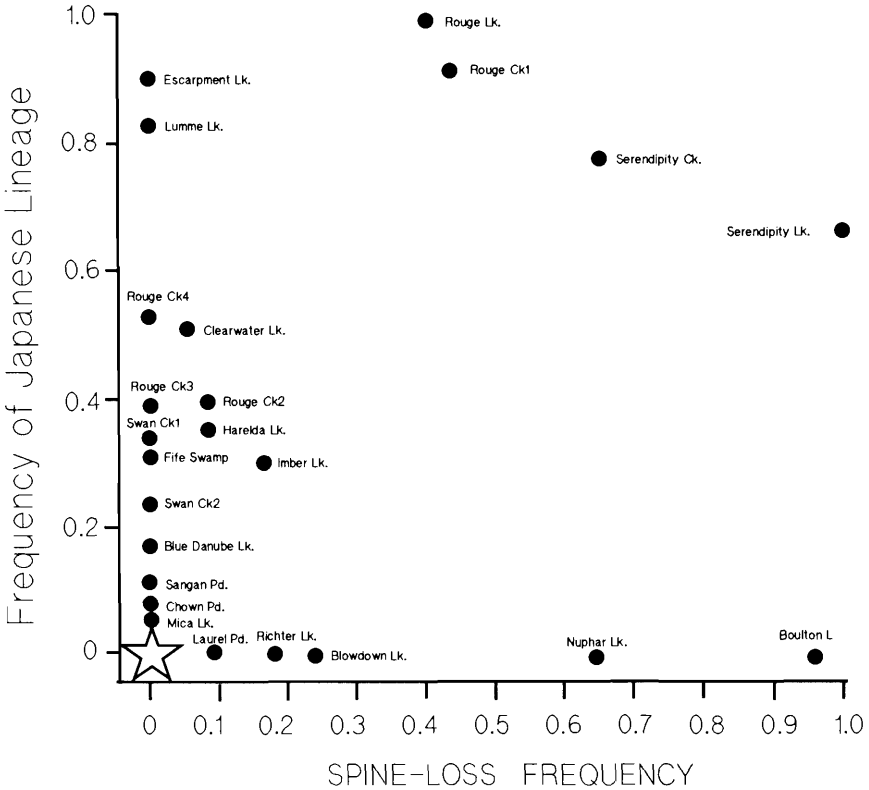


Table 1. Correlation matrix for DNA lineage and population parameters.

	DNA	LNDIS	POPSL	SAMPSL	BD50	SP50	SPLOSS	PLATE
DNA	1.00	−0.03	0.17	0.14	−0.06	−0.38*	0.41*	−0.21
LNDIS		1.00	0.47*	0.41*	−0.25	−0.13	0.11	−0.53*
POPSL			1.00	0.89*	−0.16	−0.03	0.08	−0.31
SAMPSL				1.00	0.00	0.00	0.03	−0.24
BD50					1.00	0.43*	−0.26	0.32
SP50						1.00	−0.63*	0.46*
SPLOSS							1.00	−0.20
PLATE								1.00

Note: DNA, percent occurrence of the Japanese lineage in the sample; LNDIS, natural logarithm of the distance to marine waters along the stream; POPSL, population standard length; SAMPSL, sample standard length; BD50, adjusted body depth; SP50, adjusted pelvic spine length; SPLOSS, incidence of spine loss; PLATE, plate number. The sample size is 87 for all comparisons.

*Significant at $P < 0.0018$, using sequential Bonferroni correction (two-tailed).

Table 2. Multiple-regression analyses of DNA lineage, marine distance, and morphology of the stickleback.

		Multiple	Adjusted		β coefficient						
	N	R	R^2	F^a	LNDIS	POPSL	SAMPSL	BD50	SP50	SPLOSS	PLATE
Total sample	87	0.41	0.16	17.3**	−0.07	0.13	0.13	0.05	−0.20	0.41**	−0.13
Mixed populations	19	0.75	0.53	21.6**	−0.05	0.36	0.24	−0.75**	−0.02	0.04	−0.03
White Creek watershed	8	0.92	0.83	33.8**	−0.16	−0.36	0.02	−0.32	−0.06	0.42	−0.92**

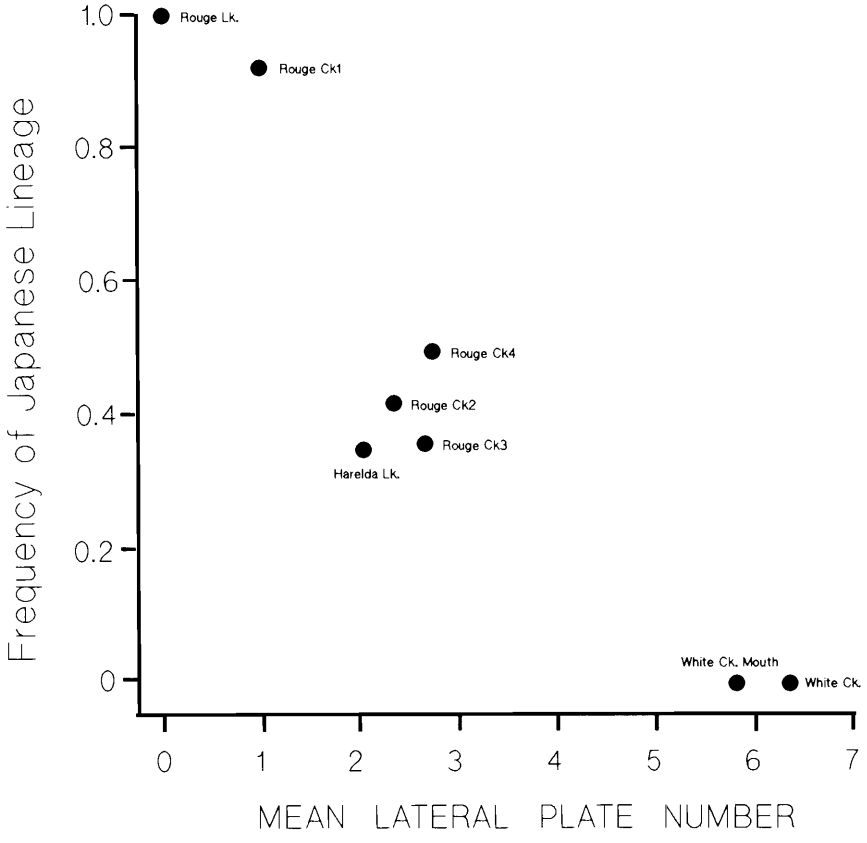
Note: For an explanation of abbreviations of morphological traits see Table 1. N is the number of populations.

[a]From ANOVAs.

*$P < 0.05$.

**$P < 0.01$.

Fig. 6. Scatterplot of the frequency of the Japanese mtDNA lineage (DNAJP) against mean lateral plate number among samples in the White Creek watershed.



variance. Absence of lateral plates, which was identified in the preliminary mtDNA survey as a characteristic of the Japanese lineage, is in fact found in both lineages. Three of the localities in which plates were completely absent were monomorphic for the Euro-North American lineage. Although two of these localities had the Japanese lineage elsewhere in the watershed, one (Slim Lake) was geographically isolated from other freshwater populations.

We obtained samples from eight localities in the White Creek watershed. Over a distance of 5 km, frequencies of Japanese haplotypes ranged from 0% at the mouth to 100% in the headwaters at Rouge Lake. The five variables accounted for 83% of the total variance in haplotype frequencies (Table 2), but only one variable, PLATE, contributed a significant component to the regression. A scatterplot of DNA against plate number is shown in Fig. 6.

If the lineages are reproductively isolated, it is reasonable to expect that a morphological signature would be evident within localities containing both lineages. Our sample sizes are small and this limits any substantive analyses. Overall, there were 95 possible comparisons between lineage and morphology among the 19 localities. Use of sequential Bonferroni correction for multiple tests indicates that none of the 95 comparisons reached the required table-wide significance level ($P < 0.0002$) (Table 3).

Discussion

mtDNA phylogeography

The presence of the two divergent stickleback mtDNA lineages on the QCI results from a combination of historical processes. The first is the origin of the two mtDNA lineages, and we will follow the suggestion by Ortí et al. (1994) that these groups diverged early in the Pleistocene during separation of the stickleback populations on either side of the Pacific Ocean. The second is the more recent event that led to the present-day distribution of these two lineages on the QCI. In a previous study involving 12 localities (O'Reilly et al. 1993), the Japanese lineage was observed only from the Argonaut Plain in the northeast region of Graham Island, and it was absent from five widely dispersed lakes elsewhere in the archipelago. In the present study, involving 87 populations, we find that stickleback with the Japanese lineage are largely, but not exclusively, restricted to a small geographical area on the Argonaut Plain. More significantly, we have also identified the lineage in two disjunct localities on the west coasts of Graham Island and Moresby Island.

The cluster of populations containing the Japanese lineage in the northeast corner of Graham Island is surrounded by populations in which only the Euro-North American lineage was detected. This distribution suggests that the Japanese lineage represents either a group of relict populations or is the result of a single source population that dispersed into a restricted group of watersheds (e.g., Bernatchez and Dodson 1991). Based on radiocarbon dates, Rouge and Serendipity ponds, which both contain high frequencies of the Japanese lineage, are between 5000 and 9500 years old (Warner 1984; R.W. Mathewes, personal communication) and therefore are unlikely candidates for a refugium. In fact, most of the northeast region of Graham Island is a broad low-lying plain covered in Late Pleistocene glacial outwash gravels, so it is

Table 3. Nonparametric analyses of DNA lineage and stickleback morphology for localities with both lineages.

Locality	N^a		Wilcoxon's rank-sum exact P value				
	ENA	JP	SL	BD50	SP50	SPLOSS	PLATE
Harelda L.	13	7	0.07+	0.24−	0.06−	0.82+	0.35−
Rouge Ck. 1	1	11	0.17+	0.67+	0.17−	0.67+	0.83−
Rouge Ck. 2	7	5	0.05+	0.07+	0.15−	0.64+	0.53−
Rouge Ck. 3	14	8	0.27+	0.48−	0.86−	1.00	0.57−
Rouge Ck. 4	3	3	0.70−	0.70+	0.20+	1.00	1.00+
Imber L.	10	4	0.23−	0.79+	0.02+	0.73+	0.95+
Serendipity L.	4	8	0.07−	0.32+	0.07+	1.00	0.81+
Serendipity Ck.	3	9	0.73−	0.86−	0.67−	1.00	0.73+
Lumme L.	2	10	0.61+	0.18−	0.91−	1.00	1.00
Swan Ck. 1	8	4	0.68+	0.93+	0.93−	1.00	1.00
Swan Ck. 2	9	3	0.73+	0.60−	0.48−	1.00	0.73+
Clearwater L.	17	17	0.52+	0.25−	0.62−	0.79−	0.79−
Fife Swp.	8	4	0.57+	0.11−	0.05−	1.00	0.93+
Mica L.	11	1	0.33+	0.67−	0.83+	1.00	0.83+
Blue Danube Ck.	6	1	1.00	0.86+	0.86+	1.00	0.86−
Chown Ck.	11	1	0.50−	1.00+	0.50−	1.00	0.33+
Sangan Pd.	11	1	0.67+	0.83+	0.67−	1.00	1.00
Gudal L.	10	1	1.00	0.89+	0.67+	1.00	0.91+
Escarpment L.	1	8	1.00	0.44−	0.89+	1.00	0.67+

Note: Morphological traits are defined in Table 1. Plus and minus signs show the directionality of morphological differences, indicating higher and lower rank means for the Japanese lineage, respectively. None of the probabilities reached table-wide significance levels ($P < 0.0002$) calculated with the sequential Bonferroni test.

[a]N is the sample size for the Euro-North American (ENA) and Japanese (JP) lineage.

highly probable that lakes in this area have developed since the last glaciation. However, 50 km to the east, proglacial freshwater lake deposits that formed during the last glacial maximum have been found in cores from the bottom of the shallow Hecate Strait, which now separates the QCI from the mainland (Josenhans et al. 1993). During the last glacial maxima, the straits would have been a broad subaerially exposed plain with scattered lakes, and these may have provided the refuge for stickleback that subsequently dispersed onto the Argonaut Plain. The presence of ancient subsurface river channels and canyons flowing eastward from the Argonaut Plain into the Hecate Strait (Fladmark 1979) indicates that fish could have had access to the Argonaut Plain during recession of the ice in the Late Pleistocene or Early Holocene (see Warner et al. 1982). This raises the question of why the populations on the east side of Graham and Moresby islands, other than at the northern tip, have the Euro-North American lineage (see Fig. 4). Current studies of the fine-scale bathymetry of the Hecate Strait region (H.W. Josenhans, personal communication) will resolve Late Pleistocene drainage channels and may illuminate the dispersal of molecular lineages on the eastern watersheds of the archipelago.

The occurrence of stickleback with the Japanese lineage on the western edge of the QCI does not conform to the glacial relict hypothesis. The west coast of the QCI is entirely mountainous, with high-gradient watersheds all draining westward into the Pacific Ocean. These watersheds have been glaciated (Sutherland-Brown and Nasmith 1962; Fedje 1993) and sediment cores at Escarpment Lake indicate a basal date near 14 000 years BP (D.W. Fedje, personal communication). Postglacial colonization appears to be the most plausible explanation for the origin of these populations. Although the Japanese lineage appears to be absent in coastal marine habitats in the QCI (O'Reilly et al. 1993; present study), populations with the Japanese lineage have recently been detected in Alaska (M.A. Bell, personal communication) and could be present in low frequencies in marine waters near the QCI.

Threespine stickleback are generally considered to be coastal in distribution (Wootton 1984), but these small fish have also been captured in the open Pacific Ocean midway between North America and Asia (Quinn and Light 1989). The origin of these stickleback is unknown but presumably they represent oceanic drift. The Alaskan Stream, which flows westward along the Aleutian Islands, has southern branches joining the easterly flow of the trans-Pacific Subarctic Current. This might carry Alaskan stickleback into the central Pacific and then back to the coast of North America. However, in addition, the Oyashio Current, which flows past Japan, joins the Subarctic Current across the Pacific to the coast of North America (Thomson 1981). Even excluding active migration, passive drift of Japanese stickleback across the Pacific could occur in 18 months (Thomson 1981), well within the 2- to 8-year life-span observed in stickleback (Wootton 1976; Reimchen 1992*b*; Baker 1994).

Our stickleback sample from the mid-Pacific demonstrates the presence of both mtDNA lineages. This increases the possibility of trans-Pacific dispersal to coastal waters of the QCI and further suggests that a glacial refugium need not

be invoked to account for the occurrence of the Japanese lineage in northwest North America (also, see Bell et al. 1993). These data also raise the question of how the two lineages could have diverged in the Early Pleistocene (Ortí et al 1994), as there appears to be evidence for extensive ocean mixing of populations.

The possibility that a refugial area did exist in the Hecate Strait region between the QCI and the continent cannot, however, be discarded on the basis of the data on stickleback mtDNA. Examination of the molecular phylogeography of other endemic taxa from the region tends to support the refugial hypothesis. MtDNA studies of the song sparrow (*Melospiza melodia*), black bear (*Ursus americanus*), and pine marten (*Martes americana*) in western Canada all show a major phylogenetic break between coastal and continental populations (Zink and Dittmann 1993; A. Byun and T.E. Reimchen, unpublished data). More molecular studies and continued work on the geological history of Hecate Strait will help clarify the glacial history and historical biogeography of the area.

Connection between morphology and mtDNA

All stickleback populations on the QCI with major loss of armour occur on the Argonaut Plain and these are geographically interspersed with typical armoured forms (Reimchen 1992*a*). Among the 12 populations examined by O'Reilly et al. (1993), each of the four population samples that contained fish belonging to the Japanese lineage had major reduction in lateral plates. In the current study, which is composed of samples from throughout the archipelago including the majority of localities on the Argonaut Plain, we have found further examples of this association between armour reduction and the presence of the Japanese lineage as well as an association with reduced relative body depth.

There are exceptions to this association. One of the armoured population samples from the west coast of Moresby Island had predominantly the Japanese lineage. Secondly, in scattered localities on the Argonaut Plain, major reduction in armour, as well as reduced body depth, also occurred in population samples containing only the Euro-North American lineage. Furthermore, our mid-Pacific samples with the Japanese lineage are fully armoured, as are marine stickleback from coastal waters near Japan (Taniguchi et al. 1990). These associations indicate that the putative ancestor of the QCI populations belonging to the Japanese lineage was armoured and that armour reduction is derived following colonization of freshwater habitats.

The complete loss of lateral plates is exceptionally uncommon in threespine stickleback, occurring in geographically diverse locations that include the Outer Hebrides (United Kingdom), California, Texada Island, and the QCI (see review in Wootton 1984). There is general consensus that this derived feature has evolved independently in these major geographical regions from the armoured source populations that characterize marine waters of the North Pacific and North Atlantic (Moodie and Reimchen 1976*b*; Reimchen 1980; Bell 1984; McPhail 1994). That the unarmoured condition is so rare globally suggests that within each geographical region, it is probably monophyletic. The geographical concentration of unarmoured forms on the Argonaut Plain is consistent with this, yet our mtDNA data show that loss of lateral plates and loss of spines occurred in populations that were monomorphic for the Japanese lineage (Rouge Lake) and monomorphic for the Euro-North American lineage (Boulton Lake, Slim Lake), highly suggestive of an independent origin of the derived morphology even within this small area. Developmental patterns of pelvic reduction in Boulton Lake stickleback are highly distinguishable from those in Serendipity Lake stickleback (dimorphic for the Japanese and Euro-North American lineages), which is consistent with an independent origin (Bell 1987).

In the White Creek drainage, there is a morphological and molecular cline from the headwaters (Rouge Lake), where the fish are unarmoured and are monomorphic for the Japanese mtDNA lineage, to the stream mouth, where the fish are armoured and are monomorphic for the Euro-North American lineage. The morphological adaptations of stickleback found in the lake differ substantively from the adaptations of those found in these streams (Reimchen et. al 1985; Reimchen 1994), therefore the major morphological differences in this watershed may simply comprise adaptations to the two habitats. The mtDNA lineage monomorphism of the Rouge Lake stickleback suggests there is little, if any, gene flow from the stream to the lake, perhaps because of the 1-m waterfall at the outlet of the lake or, possibly, reproductive isolation. Emigration out of the lake can occur and the congruent molecular and morphological cline shifting from a "lake form" to a "stream form" at progressively greater distances from the lake is consistent with genetic mixing and downstream gene flow. Without other factors operating, this should eventually lead to replacement of the Euro-North American lineage in the stream. If this is so, the current pattern may simply be transient. It is equally possible that the cline is stable. Marine and freshwater stickleback can hybridize in contact zones (for example, see Hagen 1967). Because the Euro-North American lineage is the only form currently detected in coastal estuarine waters around the QCI, this could provide a recurring source of this lineage into the White Creek drainage, which would counter the tendency towards fixation of the Japanese lineage. The resulting molecular cline would represent a combination of migration and gene flow, while the morphological cline could be reinforced by a selective gradient along the stream channel.

The occurrence of both lineages in streams might result from gene flow, but the persistence of both lineages in nine small lakes around the QCI is unexpected. Lineage sorting is expected to occur rapidly in small populations (Avise et al. 1984), and this should have occurred in these lakes, which are small (< 3.0 h) with low productivity (Reimchen 1992*a*). The two lineages could coexist if they were reproductively isolated. This has been observed among parapatric populations in this taxon (Moodie 1972; Reimchen et al. 1985; Blouw and Hagen 1990; Schluter and McPhail 1992) and is associated with morphological and ecological differentiation. Our sample sizes were small and would not have resolved fine-scale morphological differences that might exist between sibling species, but in our study we detected no statistical differences between the two lineages within the dimorphic populations. We have no current explanation for the persistence of the two lineages and have begun analyses of nuclear genes to provide insight into this issue.

The extensive morphological variation of threespine stickle-

back on the QCI provides diverse opportunities for evaluating historical and selective processes in population differentiation. That these populations were founded by two distinctive genetic lineages might account for some of the diversification among the populations. Yet the current data on the distribution of the lineages and the morphological variability in each suggest that derived traits, such as armour reduction, have arisen on multiple occasions in each of the lineages, indicating that local ecological factors (Reimchen 1980; Giles 1983; Bell et al. 1993; Bourgeois et al. 1994; Ziuganov and Zotin 1996) have the predominant role in shaping the differentiation in these populations.

Acknowledgments

We thank A. Byun, B. Koop, A. McArthur, and R. Thomson for discussion and M.A. Bell for comments on the manuscript. We also are grateful to R. Thomson, N. Davis, K. Myers, and K. Nagasawa for their assistance in acquiring stickleback from the mid-Pacific and to J. Deagle, C. Bellis, P. Hamel, and K. Thorgeirson for field assistance. This work was supported by Natural Sciences and Engineering Research Council of Canada Operating Grant A2354 to T.E.R.

References

Avise, J.C., Neigel, J.E., and Arnold, J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. **20**: 99–105.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., and Saunders, N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. **18**: 489–522.

Baker, J.A. 1994. Life history variation in the female threespine stickleback. *In* The evolutionary biology of the threespine stickleback. *Edited by* M.A. Bell and S.A. Foster. Oxford University Press, Oxford. pp. 144–187.

Bell, M.A. 1984. Evolutionary phenetics and genetics: the threespine stickleback, *Gasterosteus aculeatus*, and related species. *In* Evolutionary genetic of fishes. *Edited by* B.J. Turner. Plenum Press, New York. pp. 431–528.

Bell, M.A. 1987. Interacting evolutionary constraints in pelvic reduction of threespine sticklebacks, *Gasterosteus aculeatus* (Pisces, Gasterosteidae). Biol. J. Linn. Soc. **31**: 347–382.

Bell, M.A., and Foster, S.A. 1994. Introduction to the evolutionary biology of the threespine stickleback. *In* The evolutionary biology of the threespine stickleback. *Edited by* M.A. Bell and S.A. Foster. Oxford University Press, Oxford. pp. 1–27.

Bell, M.A., Ortí, G., Walker, J.A., and Koenings, J.P. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution, **47**: 906–914.

Bernatchez, L., and Dodson, J.J. 1991. Phylogeographic structure in mitochondrial DNA of Lake Whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution, **45**: 1016–1035.

Blouw, D.M., and Hagen, D.W. 1990 Breeding ecology and evidence of reproductive isolation of a widespread stickleback fish (Gasterosteidae) in Nova Scotia, Canada. Biol. J. Linn. Soc. **39**: 195–217.

Bourgeois, J.F., Blouw, D.M., Koenings, J.P., and Bell, M.A. 1994. Multivariate analysis of geographic covariance between phenotypes and environments in the threespine stickleback, *Gasterosteus aculeatus*, from Cook Inlet area, Alaska. Can. J. Zool. **72**: 1497–1509.

Doyle, J.J., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11–15.

Fedje, D.W. 1993. Sea-levels and prehistory in Gwaii Haanas. M.A. thesis, University of Calgary, Calgary, Alberta.

Fladmark, K.R. 1979. Routes: alternate migration corridors for early man in North America. Am. Antiq. **44**: 55–69.

Foster, J.B. 1965. The evolution of the mammals of the Queen Charlotte Islands, British Columbia. B.C. Prov. Mus. Occas. Pap. No. 14. pp. 1–30.

Gach, M.H., and Reimchen, T.E. 1989. Mitochondrial DNA patterns among endemic stickleback from the Queen Charlotte Islands: a preliminary survey. Can. J. Zool. **67**: 1324–1328.

Giles, N. 1983. The possible role of environmental calcium levels during the evolution of phenotypic diversity in outer Hebridean populations of the threespine stickleback. J. Zool. (1965–1984), **199**: 535–544.

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

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

Josenhans, H.W., Barrie, J.V., Conway, K.W., Patterson, R.T., Mathewes, R.W., and Woodworth, G.J. 1993. Surficial geology of the Queen Charlotte Basin: evidence of submerged proglacial lakes at 170 m on the continental shelf of western Canada. *In* Current research. Part A. Geological Survey of Canada, Paper 93-1A. pp. 119–127.

Kavanaugh, D.H. 1980. Insects of western Canada, with special reference to certain Carabidae (Coleoptera): present distribution patterns and their origins. Can. Entomol. **12**: 1129–1144.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., and Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. **86**: 6196–6200.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. *In* The evolutionary biology of the threespine stickleback. *Edited by* M.A. Bell and S.A. Foster. Oxford University Press, Oxford. pp. 400–447.

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

Moodie, G.E.E., and Reimchen, T.E. 1973. Endemism and conservation of sticklebacks in the Queen Charlotte Islands. Can. Field-Nat. **87**: 173–175.

Moodie, G.E.E., and Reimchen, T.E. 1976*a*. Glacial refugia, endemism and stickleback populations of the Queen Charlotte Islands. Can. Field-Nat. **90**: 471–474.

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

Moran, P., and Kornfield, I. 1993. Retention of an ancestral polymorphism in the mbuna species flock (Teleostei: Cichlidae) of Lake Malawi. Mol. Biol. Evol. **10**: 1015–1029.

Norusis, M.J. 1993. SPSS for Windows, release 6. SPSS Inc., Chicago.

Ogilvie, R.T. 1989. Disjunct vascular flora of northwest Vancouver Island in relation to Queen Charlotte Islands endemism and Pacific coast refugia. *In* The outer shores. *Edited by* G.G.E. Scudder and N. Gessler. Queen Charlotte Islands Museum Press, Skidegate, B.C. pp. 127–130.

O'Reilly, P., Reimchen, T.E., Beech, R., and Strobeck, C. 1993. Mitochondrial DNA in *Gasterosteus* and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution, **47**: 678–684.

Ortí, G., Bell, M.A., Reimchen, T.E., and Meyer, A. 1994.

Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. Evolution, **48**: 608–622.

Quinn, T.P., and Light, J.T. 1989. Occurrence of threespine sticklebacks (*Gasterosteus aculeatus*) in the open North Pacific Ocean: migration or drift? Can. J. Zool. **67**: 2850–2852.

Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predation? Can. J. Zool. **58**: 1232–1244.

Reimchen, T.E. 1983. Structural relationships between spines and lateral plates in threespine stickleback *Gasterosteus aculeatus*. Evolution, **37**: 931–946.

Reimchen, T.E. 1984. Status of unarmored and spine-deficient populations of threespine stickleback, *Gasterosteus* sp., on the Queen Charlotte Islands, British Columbia. Can. Field-Nat. **98**: 120–126.

Reimchen, T.E. 1992*a*. Naikoon Provincial Park, Queen Charlotte Islands: biophysical data for freshwater habitats. British Columbia Ministry of the Environment, Victoria.

Reimchen, T.E. 1992*b*. Extended longevity in a large-bodied *Gasterosteus* population. Can. Field-Nat. **106**: 122–125.

Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. *In* Evolutionary biology of the threespine stickleback. *Edited by* M.A. Bell and S.A. Foster. Oxford University Press, Oxford. pp. 240–276.

Reimchen, T.E., and Buckland-Nicks, J. 1990. A novel association between an endemic stickleback and a parasitic dinoflagellate. 1. Seasonal cycle and host response. Can. J. Zool. **68**: 667–671.

Reimchen, T.E., Stinson, E.S., and Nelson, J.S. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Can. J. Zool. **63**: 2940–2951.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Schluter, D., and McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. **140**: 85–108.

Schofield, W.B. 1989. Structure and affinities of the bryoflora of the Queen Charlotte Islands. *In* The outer shores. *Edited by* G.G.E. Scudder and N. Gessler. Queen Charlotte Islands Museum Press, Skidegate, B.C. pp. 109–120.

Sutherland-Brown, A., and Nasmith, H. 1962. The glaciation of the Queen Charlotte Islands. Can. Field-Nat. **76**: 209–219.

Taniguchi, N., Honma, Y., and Kawamata, K. 1990. Genetic differentiation of freshwater and anadromous threespine sticklebacks (*Gasterosteus aculeatus*) from northern Japan. Jpn. J. Ichthyol. **37**: 230–238.

Taylor, R.L. 1989. Vascular plants of the Queen Charlotte Islands. *In* The outer shores. *Edited by* G.G.E. Scudder and N. Gessler. Queen Charlotte Islands Museum Press, Skidegate, B.C. pp. 121–126.

Thomson, R.E. 1981. Oceanography of the British Columbia Coast. Can. Spec. Publ. Fish. Aquat. Sci. No. 56.

Warner, B.G. 1984. Late Quaternary paleoecology of eastern Graham Island, British Columbia. Ph.D. thesis, Simon Fraser University, Burnaby, B.C.

Warner, G.W., Mathewes, R.W., and Clague, J.C. 1982. Ice-free conditions on the Queen Charlotte Islands, British Columbia, at the height of late Wisconsin glaciation. Science (Washington, D.C.), **218**: 675–677.

Wootton, R.J. 1976. The biology of the sticklebacks. Academic Press, London.

Wootton, R.J. 1984. A functional biology of sticklebacks. University of California Press, Berkeley and Los Angeles.

Zink, R.M., and Dittmann, D.L. 1993. Geneflow, refugia and evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution, **47**: 717–729.

Ziuganov, V.V., and Zotin, A.A. 1996. Pelvic girdle polymorphism and reproductive barriers in the ninespine stickleback *Pungitius pungitius* L. from northwest Russia. Behaviour, **132**: 1095–1105.