Evolution, 47(2), 1993, pp. 678–684

MITOCHONDRIAL DNA IN *GASTEROSTEUS* AND PLEISTOCENE GLACIAL REFUGIUM ON THE QUEEN CHARLOTTE ISLANDS, BRITISH COLUMBIA

P. O'Reilly, T. E. Reimchen,[1] R. Beech, and C. Strobeck
Department of Zoology, University of Alberta, Edmonton, AB T6G 2E9, CANADA

Key words.—Evolutionary rate, *Gasterosteus*, mitochondrial DNA, phylogeny, Pleistocene refugia, Queen Charlotte Islands, zoogeography.

Received October 4, 1991. Accepted June 12, 1992.

Freshwater populations of threespine stickleback (*Gasterosteus aculeatus*) along the coast of western North America exhibit extensive morphological divergence from marine conspecifics, the presumed ancestors to freshwater populations (Hagen and McPhail, 1970; Bell, 1976). Marine sticklebacks are characterized by a complete series of bony lateral plates (30 to 35 per side) and prominent dorsal and pelvic spines. Typically, freshwater sticklebacks resemble the marine form, but can be distinguished by a deeper, slightly smaller body, and fewer lateral plates (usually three to eight per side). On the Queen Charlotte Islands (QCI), an archipelago 80 kilometers off the coast of British Columbia, Canada, freshwater populations of stickleback have been found that have highly derived morphological characters. These include gigantism in body size, complete loss of lateral plates and loss of dorsal and pelvic spines (Moodie, 1972; Moodie and Reimchen, 1976*a*; Reimchen, 1980, 1984; Reimchen et al., 1985). Populations with modest divergence in one or more of these traits occur elsewhere in the circumboreal distribution of the species including California (Miller and Hubbs, 1969), Alaska (Francis et al., 1986) and the Outer Hebrides (Campbell, 1979).

Geological evidence suggests that much of the Pacific coast of North America, north of the coterminous United States, was under the Cordilleran Ice Sheet during the last (Fraser) glaciation (Flint, 1971; Clague, 1989*a*). Since ice sheets extended well beyond present day shorelines, almost to the edge of the continental shelf (Dyke and Prest, 1987; Blaise et al., 1990), coastal islands, including the QCI, were presumed to be devoid of significant Pleistocene biological refugia (Sutherland-Brown, 1968; Sutherland-Brown and Nasmith, 1962). Morphological differentiation between marine sticklebacks and some of the highly distinctive freshwater forms was therefore ascribed to rapid post-glacial evolutionary divergence (Moodie and Reimchen, 1976*b*).

A suite of unique biological features on the QCI have led to suggestions that parts of the islands were ice free during the last glacial advance (Calder and Taylor, 1968). Endemism occurs in QCI birds and mammals (McCabe and Cowan, 1945; Foster, 1965), crustaceans (Bousfield, 1958) and insects (Kavanaugh, 1980). There are disjunct distributions of plants. Several vascular plant species are found only on the QCI and northwest Vancouver Island (Ogilvie, 1989), parts of which are also suspected of having escaped glaciation (Heusser, 1960). Many bryophytes occur within the QCI and western Europe or southeast Asia, but not elsewhere within the western hemisphere (Schofield, 1969, 1989). These patterns of distribution are presumed to result from large-scale extirpation of widely distributed species apart from local pockets that persisted in ice-free habitats (Heusser, 1989; Taylor, 1989).

Recent radiocarbon analyses and plant macrofossil data from Graham Island in northern QCI provide additional support for a refugium. At Cape Ball, on western Graham Island (Fig. 1), Warner et al. (1982) have shown that deglaciation and recolonization of aquatic and terrestrial floral communities occurred by 16,000 years B.P. As this is close to the glacial maximum on the adjacent mainland, source communities must have persisted on or close to Cape Ball for the site to have been so rapidly colonized. Isostatic depression in the vicinity of Graham Island was considerably less than on the adjacent mainland, an indication of a thin localized ice cover in the area (Warner et al., 1982).

Patterns of mitochondrial DNA (mtDNA) diversity in freshwater fishes are being examined with increasing frequency (for example, Bermingham and Avise, 1986; Billington and Hebert, 1988; Bentzen et al., 1989; Bernatchez and Dodson, 1991; Meyer et al., 1990) and most species exhibit some geographic structuring of the mtDNA phylogenetic assemblages. In these studies, major genetic breaks and their times of divergence have been associated with historical vicariant events, such as watershed isolation or coalescence, Pleistocene glaciation or lake formation (for review, see Avise et al., 1987*a*). In a preliminary study of mtDNA variation in threespine sticklebacks from central Graham Island, QCI, high similarity was observed between several morphologically divergent populations and a sample of marine sticklebacks, consistent with a post-glacial origin of the freshwater populations (Gach and Reimchen, 1989). In the present study, we expand this original survey to encompass nine endemic freshwater populations from throughout the QCI and compare these with sticklebacks from the surrounding marine habitat, the adjacent mainland and the Atlantic Ocean. Our objective was to examine patterns and approximate times of stickleback colonization in an effort to

[1] Author to whom correspondence should be addressed and present address: Department of Biology, University of Victoria, Box 1700, Victoria, BC V8W 2Y2, Canada.

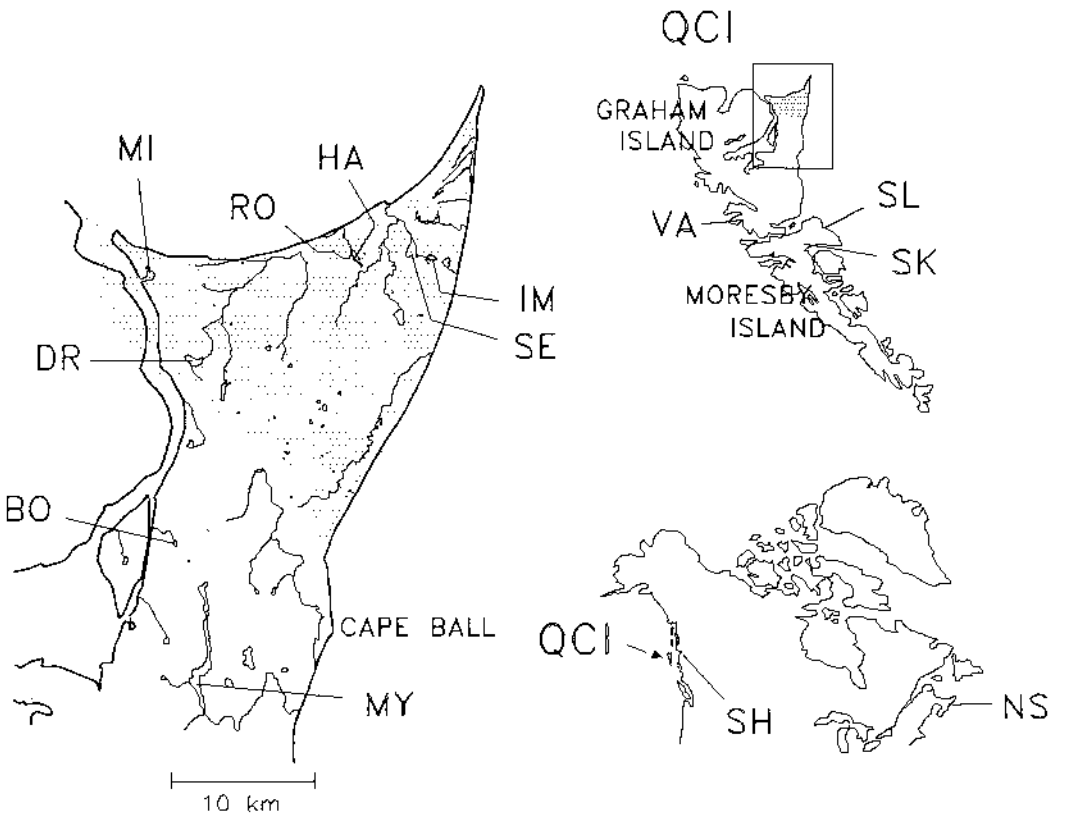


FIG. 1. Collection localities for *Gasterosteus*. Marine populations—Masset Inlet (MI), Nova Scotia (NS) and Sheldon's Lagoon (SL). Freshwater populations—Boulton Lake (BO), Drizzle Lake (DR), Harelda Lake (HA), Imber Lake (IM), Mayer Lake (MY), Rouge Lake (RO), Serendipity Lake (SE), Shrimp Lake (SR), Skidegate Lake (SK) and Van Lake (VA). Hatched area denotes glacial outwash plain (Argonaut Plain).

distinguish between pre-glacial and post-glacial colonization.

MATERIALS AND METHODS

Sampling sites are shown in Figure 1. These comprise the following: four lakes from the Argonaut Plain (Imber, Serendipity, Harelda, Rouge) having populations of sticklebacks with major reduction in spines and lateral plates (Reimchen, 1984; Reimchen, unpubl. data); two localities from central Graham Island (Drizzle Lake, Mayer Lake) which contain giant sticklebacks) (Moodie, 1972; Reimchen et al., 1985); one locality from central Graham Island (Boulton Lake) which has spine-deficient sticklebacks (Reimchen, 1980); one locality from the western coast of Graham Island (Van Lake) with undifferentiated low-plated sticklebacks (Reimchen, 1983); one locality from northern Moresby Island (Skidegate Lake) having giant sticklebacks, one locality from mainland British Columbia (Shrimp Lake) with undifferentiated low-plated sticklebacks; one marine locality from northern Graham Island (Masset Inlet) with fully plated sticklebacks; one marine locality from northern Moresby Island (Sheldon's Lagoon) with fully plated sticklebacks; and one marine locality from the Atlantic Ocean (Nova Scotia) with fully plated sticklebacks.

Adult sticklebacks were collected from each locality with minnow traps or nets and transferred live to the laboratory. Sample size ranged from 10 to 16 for each site except for Nova Scotia where only three individuals were processed. Total cellular DNA (nuclear and mitochondrial) was extracted from 147 individuals. Samples were digested with 10 restriction endonucleases (*Hin*FI, *Bgl*I, *Eco*RI, *Hin*dIII, *Pst*I, *Pvu*II, *Sst*I, *Sst*II, *Sal*I, and *Hin*cII). Due to low yields of mtDNA, 10 individuals were not surveyed with the monotypic enzyme *Sst*II and two from Van Lake were not surveyed for *Hin*dIII. Resulting fragments were separated by electrophoresis through 1% agarose gels and transferred to nylon membranes by Southern blotting techniques (Southern, 1975; Wahl et al., 1979). The entire mtDNA genome of threespine sticklebacks, cloned into the plasmid pUC19, was radioactively labeled (Rigby et al., 1977) and hybridized to membrane-bound DNA at 65°C in a solution of 7% SDS, 1.0 mM EDTA and 0.263 M Na_2HPO_4, pH 8.0 (Church and Gilbert, 1984). Membranes were washed and then exposed to X-ray film for one to three days.

Restriction site maps of all enzymes used in the survey, except *Hin*FI, which recognizes a pentanucleotide sequence, were constructed using both double and partial digestion methods (see Sambrook et al., 1989).

TABLE 1. Haplotype composition, nucleon diversity estimates, and estimates of maximum divergence from Pacific marine haplotypes for *Gasterosteus* populations. N = individuals surveyed. The largest of pairwise divergence estimates between haplotypes from a given locality and the most closely related from Pacific marine samples was used as a measure of the maximum amount of divergence of a population from Pacific marine sticklebacks.

Location (N)	Haplotype (frequency)	Nucleon diversity h	Maximum divergence from nearest marine haplotype observed
Queen Charlotte Island lakes			
Drizzle (12)	A (12)	0	0
Boulton (9)	A (7), D (1), G (1)	0.417	0.564
Mayer (11)	A (3), H (8)	0.436	0.564
Skidegate (12)	A (4), B (1), H (6), I (1)	0.682	0.868
Van (11)	C (1), F (10)	0.182	0.268
Queen Charlotte Argonaut Plain lakes			
Rouge (10)	L (10)	0	2.455
Harelda (16)	A (9), C (2), L (5)	0.608	2.455
Serendipity (15)	A (6), C (1), L (8)	0.591	2.455
Imbre (15)	A (3), B (2), C (7), K (1), L (2)	0.581	2.091
Mainland Lake			
Shrimp (12)	A (8), H (4)	0.485	0.564
Pacific marine localities			
Masset (8)	A (3), C (5)	0.536	—
Sheldon (13)	A (8), C (4), E (1)	0.564	—
Atlantic marine locality			
Nova Scotia (3)	J (3)	N/A	1.151

Restriction sites produced by a given enzyme were mapped relative to the positions of cleavage sites generated by at least two other enzymes. Of the 40 sites located, 18 were polymorphic (Fig. 2a). Since a large number of fragments were generated by *Hin*FI, these restriction sites were not mapped. Nucleotide diversity between pairs of haplotypes was estimated from mapped site differences using the maximum likelihood method of Nei and Tajima (1983).

A Wagner parsimony network of the 12 mtDNA haplotypes was constructed from site presence-absence data using PAUP (Phylogenetic Analysis Using Parsimony, version R, written by D. Swofford, Illinois Natural History Survey, Champaign, Illinois). A majority rule consensus tree and reliability statements of the branch nodes of the maximum parsimony network were estimated using the bootstrapping algorithm (with 100 replicates).

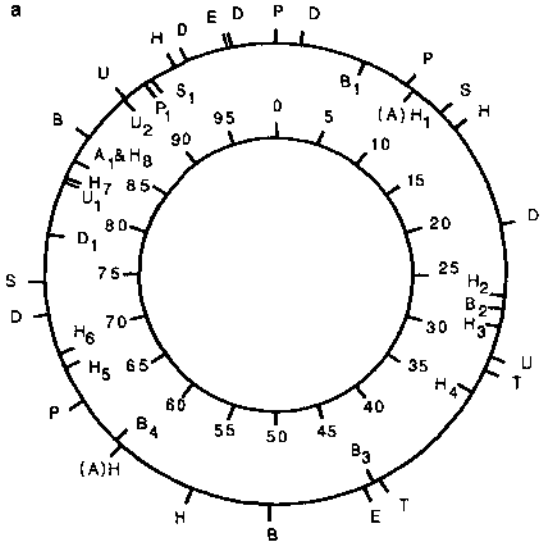
Nucleon diversity was calculated (Eq. 7, Nei and Tajima, 1981). We tested whether there was heterogeneity among Pacific populations for haplotype frequencies using RXC tests of independence. The two marine populations (Sheldon and Masset) were combined, as their frequencies were not significantly different ($G = 0.71$, $P = 0.4$). Yate's correction was applied for small sample sizes (see Sokal and Rohlf, 1981).

RESULTS AND DISCUSSION

We identified 11 mtDNA haplotypes from the Pacific region. These include a common form (43% of individuals sampled), designated as A, four haplotypes with moderate frequencies (12 to 17%) and six rare haplotypes (< 7%). Haplotype frequencies at each locality, nucleon diversity and maximum sequence divergence are shown in Table 1. Significant heterogeneity in haplotype frequencies exists among locations sampled ($G_H = 266.02$, $P < 0.001$). Nucleon diversity ranges from zero at Drizzle Lake and Rouge Lake to 0.75 at Imber Lake where there were five haplotypes among 15 individuals.

Based on restriction-site patterns, two distinct mtDNA lineages were evident in the Wagner parsimony network (Fig. 2b); this grouping was supported in 100% of the bootstrapping replicates. Lineage designations were also concordant with restriction fragment patterns generated by the pentanucleotide enzyme *Hin*FI. The first lineage is composed of nine haplotypes of which all but one can be derived from the closest haplotype by a single site change. The haplotypes were widespread, occurring in marine waters and in freshwaters from the mainland of British Columbia and from the Queen Charlotte Islands. Localities include stickleback populations that range from underived ancestral forms through to highly divergent populations with gigantism in body size (Moodie and Reimchen, 1976*a*). This assemblage of related haplotypes will be referred to as the Marine lineage. The second lineage was composed of two haplotypes (K, L) which were at least seven sites removed from the Marine lineage. These were restricted to the Argonaut Plain on the northeastern region of Graham Island (Fig. 1) and will be referred to as the Argonaut lineage.

Pairwise estimates of sequence divergence between Argonaut and Marine haplotypes averaged 2.49% (2.09 to 2.84%). Using the rate of mtDNA sequence divergence calibrated for mammals of 2% per million years



b

Restriction Site Pattern

	Type	Location(s)	Freq. (%)	B_1	B_2	B_3	B_4	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	D_1	P_1	U_1	U_2	S_1	A_1	Restriction Fragment Pattern
marine	J	Ns	2	-	+	-	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	I
	I	Sk	1	-	+	-	-	+	+	-	+	-	-	-	+	+	-	-	-	+	+	I
	H	My, Sk, Sr	12	-	+	-	-	+	+	-	+	-	-	-	+	+	-	+	-	+	+	I
	B	Im, Sk	2	-	+	-	-	+	+	-	+	-	-	-	+	+	-	+	-	+	-	I
	E	Sl	1	-	+	-	-	+	+	-	-	+	-	-	+	+	-	+	-	+	-	I
	A	Bo, Dr, Ha, Im, Mi My, Se, Sk, Sl, Sr	43	-	+	-	-	+	+	-	+	+	-	-	+	+	-	+	-	+	-	I
	D	Bo	1	-	+	-	+	+	+	-	+	+	-	-	+	+	-	+	-	+	-	I
	C	Ha, Im, Mi, Se Sl, Va	14	-	+	-	-	+	+	-	+	+	+	-	+	+	-	+	-	+	-	I
	F	Va	7	-	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-	+	-	I
	G	Bo	1	-	+	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	I
argonaut	K	Im	1	+	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-	II
	L	Ha, Im, Ro, Se	17	+	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	II

FIG. 2. a. Positions of monomorphic (outside circle) and polymorphic (inside circle) restriction sites for enzymes *Bgl*I (B), *Eco*RI (E), *Hin*cII (H), *Hin*dIII (D), *Pst*I (P), *Pvu*II (U), *Sal*I (A), *Sst*I (S), and *Sst*II (T). The monomorphic *Pst*I restriction site at the top of the circle was arbitrarily chosen as the start of the mtDNA map. Numerical subscripts associated with each variable site are referenced in Figure 2b for defining composite site maps of all haplotypes. *Hin*cII recognizes a subset of the hexanucleotide sequence identified by *Sal*I, and two corresponding sites often changed together; in such cases *Sal*I was bracketed (A), and not incorporated into the analyses. A_1, however, behaved independently of the corresponding H_8, and was therefore treated as an additional site. b. Wagner parsimony network generated from site presence–absence data of 12 mtDNA haplotypes. Number of restriction sites shown as dashes on each branch. Numbers at select nodes show percentage of replicates from bootstrapping where haplotypes grouped together. Presence–absence states for all sites are shown corresponding to designations for restriction fragment patterns generated by the pentanucleotide enzyme *Hin*FI. Patterns I and II shared 4 of 10 fragments and differed by a minimum of three inferred restriction site changes. See Figure 1 for locality acronyms.

(Brown et al., 1979), the Argonaut and Marine lineages would have diverged 1.2 million years B.P. near the beginning of the Pleistocene. Uncertainties in calibration and differential rates of mtDNA evolution (Vawter and Brown, 1986; Moritz et al., 1987; Thomas and Beckenbach, 1989) limit confidence in this estimate, but even if the rates were an order of magnitude higher, the mean time for divergence of the Argonaut and Marine lineages would be over 100,000 years B.P., well before the last glacial advance. The absence in marine waters of the Argonaut haplotypes in addition to the absence of the seven intermediate haplotypes between the Argonaut lineage and Marine haplotypes strongly suggests that the lineage has been isolated in a freshwater refugium throughout the last glacial advances and perhaps throughout much of the Pleistocene. Furthermore, large phylogenetic discontinuities (breaks between arrays of related haplotypes) of the magnitude observed in several of the Argonaut locations have been rarely reported for conspecifics from a single collection site (see Avise et al., 1987*a*). Where observed, such patterns have been associated with secondary contact between long separated populations (Bermingham and Avise, 1986; Gonzalez-Villasenor and Powers, 1990). Morphological data show no evidence for current introgression between marine sticklebacks and the distinct forms found in the Argonaut populations (Reimchen, 1984). Possibly, genetic exchange may have occurred during the elevated sea levels about 8,000 years B.P. when submerged river valleys on the Argonaut Plain (Clague, 1989*b*) could have given marine sticklebacks access to these endemic lake populations.

Characterization of the Atlantic mtDNA haplotype (J) accentuates the divergence observed in the Queen Charlotte localities. Currently, the northern extent of *G. aculeatus* in the Pacific Ocean is the Bering Strait; in the western Atlantic, the species occurs to the northern region of Hudson Bay (Wootton, 1984) and there are no extant populations of sticklebacks across the central Arctic. Other fish species show comparable separation between Pacific and the Atlantic basins but the duration of the separation is poorly known (Herman and Hopkins, 1980; Grant and Stahl, 1988). The 8,000 km of coastline separating Nova Scotia and Queen Charlotte Island populations as well as the current discontinuity in distribution might be expected to yield mtDNA that is more divergent than the potentially overlapping populations of the Argonaut Plain. Yet the Atlantic haplotype near Nova Scotia is less divergent from the Pacific haplotypes around the Queen Charlotte Islands (1.15% divergence) than the Pacific marine haplotypes are from the Argonaut lineage (2.09% minimum divergence; see Table 1) suggesting that the Atlantic and Pacific *Gasterosteus* haplotypes have diverged more recently.

An alternative interpretation to prolonged isolation in the Argonaut Plain is post-glacial colonization by marine sticklebacks followed by rapid differentiation. The substitution rates of non-neutral mutations in small populations that have undergone repeated bottlenecks can be more rapid than in those with a very large population size (Ohta, 1973, 1976). In *Drosophila*, rates of mtDNA evolution were three times higher in small than in large populations (Desalle and Templeton, 1988). The highest frequencies of the L haplotype in Queen Charlotte *Gasterosteus* were found in the two smallest lakes (approximately 1 ha), where bottlenecks could have occurred. However, the L haplotype is found in two separate watersheds. For this hypothesis to be correct would require parallel evolution of the L haplotype, concomitant loss of intermediate haplotypes and an evolutionary rate several orders of magnitude higher than those reported in the literature (Brown et al., 1982; Hixson and Brown, 1986; Powell et al., 1986).

Pre-glacial colonization would also be refuted if the Argonaut lineage haplotypes or any of the intermediate haplotypes between the Argonaut and the Marine lineages were discovered in marine waters. That we did not detect these haplotypes in marine localities may simply reflect sampling error, a probable event if frequencies of the Argonaut or intermediate haplotypes were very low and restricted in geographical distribution. This cannot be excluded without further sampling in marine habitats. Yet there are several difficulties with this interpretation. Morphological and allozyme data demonstrate minimal population substructuring in marine sticklebacks relative to that observed in freshwater habitats (Bell, 1976; Withler and McPhail, 1985). As well, restricted spatial distribution of highly divergent mtDNA haplotypes in species inhabiting geographically continuous marine habitats would be very unusual (cf. Avise et al., 1987*a*, 1987*b*).

All of the stickleback populations with the Argonaut mtDNA lineage are characterized by major reduction of bony lateral plates and frequently, fish lack all plates (Reimchen, unpubl. data); this represents a highly derived condition for *Gasterosteus* (Bell, 1976). The population in Rouge Lake, which is monomorphic for the Argonaut haplotype, exhibits some of the most pronounced reduction in spines observed in the species (Reimchen, 1984). As well, it is symbiotic with an unusual taxon of dinoflagellate, an association which appears to have no counterpart elsewhere in the circumboreal distribution of either group (Reimchen and Buckland-Nicks, 1990; Buckland-Nicks et al., 1990). When these factors are taken in concert with the predicted divergence time derived from mtDNA, an extended pre-glacial history in the Argonaut Plain is suggested. If a refugium was large enough to support freshwater fish throughout the Pleistocene, then other aquatic and terrestrial biota may also have persisted during this period. Recognition of a divergent haplotype in any of the other endemic taxa from the region would increase confidence in the implications of the present study and if upheld, would influence paleozoogeographic reconstruction of western North America.

Acknowledgments

We thank M. Blouw for sending live stickleback from Nova Scotia, J. Barret for technical assistance, S. D. Douglas, T. E. Dowling, M. H. Gach and R. Honeycutt for comments on the manuscript and R. Mathewes for discussion. Logistical support was provided by P. Marhenke III, R. Henderson, and F. Henderson. We acknowledge the Council of the Haida Nation and the Ministry of Parks, Government of British Columbia, for permission to sample in the Drizzle Lake Ecological Reserve and Naikoon Provincial Park. Major contributions of authors to this study include project origin (T.E.R.), field collections (P.O.R.), mtDNA extraction and analysis (P.O.R.), molecular technical support

(R.B.), manuscript preparation (P.O.R., T.E.R.) and laboratory facilities (C.S.). This work was funded principally by a grant (A2354) to T.E.R. from the Natural Sciences and Engineering Research Council of Canada.

Literature Cited

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

Avise, J. C., C. A. Reeb, and N. C. Saunders. 1987*b*. Geographic population structure and species differences in mitochondrial DNA of mouthbrooding marine catfishes (Ariidae) and demersal spawning toadfishes (Batrachoididae). Evolution 41:991–1002.

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

Bentzen, P., G. G. Brown, and W. C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure and life history variation in American shad (*Alosa sapidissima*). Can. J. Fish. Aquat. Sci. 46: 1446–1454.

Bermingham, E., and J. C. Avise. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics 113:939–965.

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

Billington, N., and P. D. N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45:643–654.

Blaise, B., J. J. Clague, and R. W. Mathewes. 1990. Time of maximum late Wisconsin glaciation, west coast of Canada. Quat. Res. 34:282–295.

Bousfield, E. L. 1958. Freshwater amphipod crustaceans of glaciated North America. Can. Field Na. 72:55–113.

Brown, W. M., M. George, and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967–1971.

Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequence of primates: Tempo and mode of evolution. J. Mol. Evol. 18:225–239.

Buckland-Nicks, J. A., T. E. Reimchen, and M. F. Taylor. 1990. A novel association between an endemic stickleback and a parasitic dinoflagellate. 2. Morphology and life cycle. J. Phycol. 26:539–548.

Calder, J. A., and R. L. Taylor. 1968. Flora of the Queen Charlotte Islands. Can. Dep. Agric. Res. Br. Monogr. No. 4.

Campbell, R. N. 1979. Sticklebacks (*Gasterosteus aculeatus* [L.] and *Pungitius pungitius* [L.]) in the Outer Hebrides, Scotland. Hebridean Nat. 3:8–15.

Church, G. M., and W. Gilbert. 1984. Genome sequencing. Proc. Natl. Acad. Sci. USA 81:1991–1995.

Clague, J. J. 1989*a*. Cordilleran ice sheet, pp. 40–42. *In* R. J. Fulton (ed.), Quaternary Geology of Canada and Greenland, No. 1, Geological Survey of Canada, Geology of Canada.

———. 1989*b*. Quaternary geology of the Queen Charlotte Islands pp. 65–74. *In* G. G. E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Islands Museum Press, Queen Charlotte City, BC, Canada.

Desalle, R., and A. R. Templeton. 1988. Founder effects and the rate of mitochondrial DNA evolution in Hawaiian Drosophila. Evolution 42:1076–1084.

Dyke, A. S., and V. K. Prest. 1987. Paleogeography of northern North America, 18,000–12,000 years ago. Sheet 1 of 3. Geological Survey of Canada Map 1703A.

Flint, R. F. 1971. Glacial and Quaternary Geology. John Wiley and Sons, Inc., N.Y., USA.

Foster, J. B. 1965. The evolution of the mammals of the Queen Charlotte Islands, British Columbia. British Columbia Provincial Museum Occasional Papers, No. 14:1–130.

Francis, R. C., J. V. Baumgartner, A. C. Havens, and M. A. Bell. 1986. Historical and ecological sources of variation among lake populations of threespine sticklebacks, *Gasterosteus aculeatus*, near Cook Inlet, Alaska. Can. J. Zool. 64:2257–2265.

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

Gonzalez-Villasenor, L. I., and D. A. Powers. 1990. Mitochondrial-DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitus* support secondary introgradation. Evolution 44:27–37.

Grant, W. S., and G. Stahl. 1988. Evolution of Atlantic and Pacific cod: Loss of genetic variation and gene expression in Pacific cod. Evolution 42: 138–146.

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

Herman, Y., and D. M. Hopkins. 1980. Arctic oceanic climate in late Cenozoic time. Science 209: 557–562.

Heusser, C. J. 1960. Late-Pleistocene environments of North Pacific North America. Amer. Geogr. Soc. Misc. Publ. 35. 308 pp.

———. 1989. North Pacific coastal refugia—The Queen Charlotte Islands in perspective, pp. 91–106. *In* G. G. E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Islands Museum Press, Queen Charlotte City, BC, Canada.

Hixson, J. E., and W. M. Brown. 1986. A comparison of the small ribosomal RNA gene from the mitochondrial DNA of the great apes and human: Sequence, structure, evolution and phylogenetic implication. J. Mol. Biol. Evol. 3:1–18.

Kavanaugh, D. H. 1980. Insects of western Canada, with special reference to certain Carabidae (Coleoptera): Present distribution patterns and their origins. Can. Entomol. 112:1129–1144.

McCabe, T. T., and I. McT. Cowan. 1945. *Pero-*

myscus maniculatus macrorhinus and the problem of insularity. Trans. R. Can. Inst. 1945:117–215.

MEYER, A., T. D. KOCHER, P. BASASIBWAKI, AND A. C. WILSON. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347:550–553.

MILLER, R. R., AND C. L. HUBBS. 1969. Systematics of *Gasterosteus aculeatus*, with particular reference to intergradation and introgression along the Pacific Coast on North America: A commentary on a recent contribution. Copeia 1969:52–69.

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

MOODIE, G. E. E., AND T. E. REIMCHEN. 1976*a*. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Syst. Zool. 25:49–61.

———. 1976*b*. Glacial refugia, endemism, and stickleback populations of the Queen Charlotte Islands, British Columbia. Can. Field Nat. 90:471–474.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18:269–292.

NEI, M., AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:233–245.

———. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. Genetics 105:207–217.

OGILVIE, R. T. 1989. Disjunct vascular flora of northwestern Vancouver Island in relation to Queen Charlotte Islands' endemism and Pacific Coast refugia, pp. 127–130. *In* G. G. E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Islands Museum Press, Queen Charlotte City, BC, Canada.

OHTA, T. 1973. Slightly deleterious mutant substitutions in evolution. Nature 246:96–98.

———. 1976. Role of slightly deleterious mutations in molecular evolution and polymorphisms. Theor. Popul. Biol. 10:254–275.

POWELL, J. R., A. CACCONE, G. D. AMATO, AND C. YOON. 1986. Rates of nucleotide substitution in *Drosophila* mitochondrial DNA and nuclear DNA are similar. Proc. Natl. Acad. Sci. USA 83:9090–9093.

REIMCHEN, T. E. 1980. Spine-deficiency and polymorphism in a population of *Gasterosteus aculeatus*: An adaptation to predators? Can. J. Zool. 58: 1232–1244.

———. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). Evolution 37:931–946.

———. 1984. Status of unarmoured and spine-deficient populations (Charlotte unarmoured stickleback) of threespine stickleback, *Gasterosteus* sp., on the Queen Charlotte Islands, British Columbia. Can. Field Nat. 98:120–126.

REIMCHEN, T. E., AND J. A. BUCKLAND-NICKS. 1990. A novel association between an endemic stickleback and a parasitic dinoflagellate. I. Seasonal cycle and host response. Can. J. Zool. 68:667–671.

REIMCHEN, T. E., E. M. STINSON, AND J. S. NELSON. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Can. J. Zool. 63:2944–2951.

RIGBY, P. W. J., M. DIECKMANN, C. RHODES, AND P. BERG. 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113:237–251.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., USA.

SCHOFIELD, W. B. 1969. Phytogeography of northwestern North America: Bryophytes and vascular plants. Madroño 20:155–207.

———. 1989. Structure and affinities of the Bryoflora of the Queen Charlotte Islands, pp. 109–119. *In* G. G. E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Islands Museum Press, Queen Charlotte City, BC, Canada.

SOKAL, R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. Freeman, San Francisco, CA USA.

SOUTHERN, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

SUTHERLAND-BROWN, A. 1968. Geology of the Queen Charlotte Islands, British Columbia. Bull. B.C. Dept. Mines Pet. Resour. 54:1–226.

SUTHERLAND-BROWN, A., AND NASMITH. 1962. The glaciation of the Queen Charlotte Islands. Can. Field Nat. 76:209–219.

TAYLOR, R. L. 1989. Vascular plants of the Queen Charlotte Islands, pp. 121–125 *In* G. G. E. Scudder and N. Gessler (eds.), The Outer Shores. Queen Charlotte Islands Museum Press, Queen Charlotte City, BC, Canada.

THOMAS, W., AND A. T. BECKENBACH. 1989. Variation in salmonid mitochondrial DNA: Evolutionary constraints and mechanisms of substitution. J. Mol. Evol. 29:233–245.

VAWTER, L., AND W. M. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science 234: 194–195.

WAHL, G. M., M. STERN, AND G. R. STARK. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA 76:3683–3687.

WARNER, B. G., R. W. MATHEWES, AND J. J. CLAGUE. 1982. Ice-free conditions on the Queen Charlotte Islands, British Columbia, at the height of the late Wisconsin glaciation. Science 218:675–677.

WITHLER, R. E., AND J. D. MCPHAIL. 1985. Genetic variability in freshwater and anadromous sticklebacks (*Gasterosteus aculeatus*) of southern British Columbia. Can. J. Zool. 63:528–533.

WOOTTON, R. J. 1984. A Functional Biology of Sticklebacks. University of California Press, Los Angeles, CA USA.

Corresponding Editor: R. Honeycutt