Mitochondrial DNA patterns among endemic stickleback from the Queen Charlotte Islands: a preliminary survey

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Received December 30, 1987

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.

Mitochondrial DNA (mtDNA) restriction fragments were analyzed in 30 individuals from four populations of *Gasterosteus aculeatus* from the Queen Charlotte Islands, British Columbia. Two morphologically divergent endemic freshwater populations (Boulton Lake, Drizzle Lake), a typical stream form, and a brackish form were sampled. mtDNA size variation of 70 to 180 base pairs was noted among individuals from all populations, and 1.1 and 5.0 kilobase duplications were observed in mtDNAs from two individuals. Analysis of 75 mtDNA fragments produced by five restriction endonucleases revealed seven clones differing by less than 1.0% sequence divergence. While the high degree of genetic similarity is consistent with a postglacial origin of these populations, the presence of a unique restriction site among geographically isolated populations suggests that these endemics may have had a common freshwater ancestor that inhabited periglacial freshwater habitats rather than being independently derived from marine forms.

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

Les fragments de restriction de l'ADN mitochondrial (ADNmt) ont été analysés chez 30 individus de *Gasterosteus aculeatus* appartenant à quatre populations différentes des Îles de la Reine Charlotte, Colombie-Britannique : deux populations dulcicoles endémiques morphologiquement divergentes (lac Boulton, lac Drizzle), une forme typique d'eau courante et une forme d'eau saumâtre. La variation de taille des fragments allait de 70 à 180 paires de base chez les individus de toutes les populations et des duplications de 1,1 et 5,0 kilobases ont été observées dans l'ADNmt de deux individus. L'analyse de 75 fragments produits par cinq endonucléases de restriction a révélé l'existence de sept clones distincts par moins de 1,0% de divergence entre les séquences. L'importance de la similarité génétique confirme l'origine post-glaciaire de ces populations, mais la présence d'un site de restriction particulier aux populations isolées géographiquement semble indiquer que ces entités endémiques proviennent probablement d'un ancêtre dulcicole commun qui occupait les habitats d'eau douce périglaciaires et qu'elles ne dérivent pas de formes marines différentes.

[Traduit par la revue]

Introduction

Extensive endemism in Queen Charlotte Island (QCI) populations of the threespine stickleback (*Gasterosteus aculeatus*) represents either rapid postglacial evolution from marine ancestors (Moodie and Reimchen 1976*a*) or relict populations from Quaternary glacial refugia postulated for the region (Warner et al. 1982). Although the morphological variation has been well documented in many QCI stickleback populations (Moodie and Reimchen 1976*b*; Reimchen et al. 1985), the question of historical relationships of these populations has not been directly addressed. Mitochondrial DNA (mtDNA) has been used to examine population-level relationships (Moritz et al. 1987) because it exhibits haploid maternal inheritance (Avise and Lansman 1983) and rapid evolution (Brown et al. 1979, 1982; Vawter and Brown 1986). Most mtDNA substitutions are also assumed to be selectively neutral with respect to population-level differences (Clark and Lyckegaard 1988, and references therein). This characteristic is especially useful for our study because of the obvious complications introduced by the influence of selection in molding morphological characteristics of stickleback (Hagen and Gilbertson 1972; Moodie 1972; Moodie and Reimchen 1976*b*; Bell and Haglund 1978; Reimchen 1980, 1983). We report here the results of a preliminary mtDNA survey performed to examine the origin and evolution of morphologically divergent populations of threespine stickleback in the QCI. In particular, we have attempted to discover whether these endemic freshwater populations are refugial relicts, as has been suggested for other taxa in the region (Bousfield 1958; Foster 1965; Schofield 1969; Kavanaugh 1980), and whether they have been independently derived from marine populations, which is the assumed mode of evolution for coastal populations (Hagen and McPhail 1970; Bell 1976; Withler and McPhail 1985).

Methods

Individuals were collected from four localities (Fig. 1) chosen to include (*i*) a morphotypically marine population (Delkatla Estuary, $N = 7$) representative of the presumed freshwater ancestor, (*ii*) two highly endemic forms (Boulton Lake, $N = 12$; Drizzle Lake, $N = 4$) found in isolated watersheds, and (*iii*) a stream population (Drizzle Outlet, $N = 6$) parapatric with the Drizzle Lake form but considered to be reproductively isolated from it (Reimchen et al. 1985). Further

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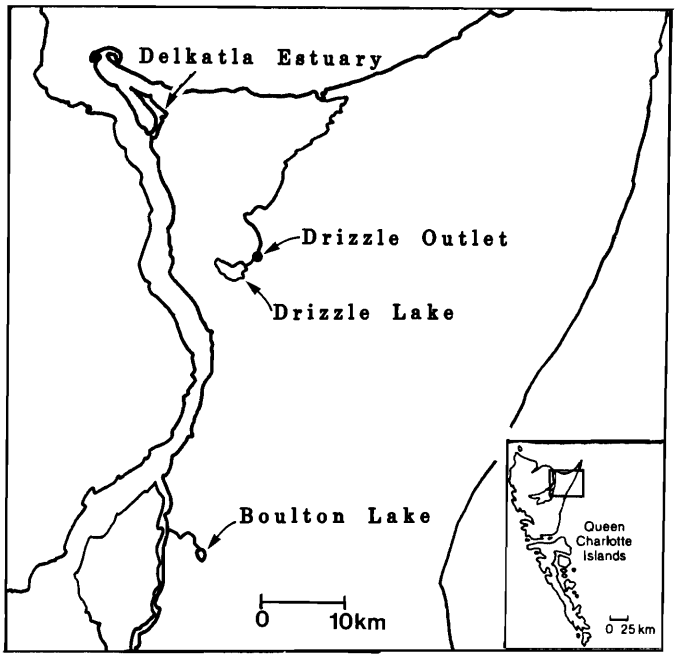

FIG. 1. Collection localities on Graham Island, Queen Charlotte Islands.

descriptions of the habitat and broad morphological characters of the *Gasterosteus* found in Boulton Lake, Drizzle Lake, and Drizzle Outlet are given elsewhere (Reimchen 1980; Reimchen et al. 1985). Delkatla Estuary is a brackish 1-ha pond at the edge of a marine estuary. The pond was within the upper reaches of spring tides until 1964, when tidal flow was reduced following construction of a dyke across much of the entrance to the estuary. The pond currently has low conductivity (332 μS cm^{-1}) but is still subject to brackish water incursion during extreme spring tides.

Mitochondrial DNA was purified from pooled organs (liver, kidney, heart, and gonad) of each individual, following Wright et al. (1983) and Densmore et al. (1985). Tissue was homogenized in 10 mL of 0.25 M sucrose, 10 mM Tris, 100 mM EDTA, pH 7.5. mtDNA was separated from nuclear DNA by sedimentation equilibrium centrifugation in cesium chloride – propidium iodide gradients. Aliquots of each purified mtDNA sample were digested separately with each of five restriction endonucleases (*Ava*I, *Eco*RI, and *Hin*dIII, which recognize specific sequences of six nucleotide bases; *Mbo*I and *Hin*fI, with four-base recognition sequences). Following digestion, mtDNA fragments were end-labeled (Brown 1980) and separated according to size by overnight electrophoresis on 1.2% agarose and 4.0% polyacrylamide gels. Fragment length was determined by comparison with known standards (λ phage DNA digested with *Hin*dIII and ϕX RF DNA digested with *Hae*III for agarose and polyacrylamide gels, respectively). A composite digestion profile was constructed for each individual from the *Eco*RI, *Mbo*I, and *Hin*fI profiles. No variation was detected with *Hin*dIII and *Ava*I. Population mtDNA composition was examined with Fisher's exact test (Dixon and Massey 1969).

Samples of stickleback collected during earlier population studies (Reimchen 1980; Reimchen et al. 1985) were used for morphological analyses. Male adult stickleback were measured for the following traits: standard length, body depth, pelvic spine length, length and width of posterior process of pelvic skeleton, length of ascending branch of pelvic skeleton, pectoral fin length, length of orbit, height of seventh lateral plate, number of lateral plates left side, number of dorsal spines, number of pelvic spines, presence or absence of anal spine (terminology and description in Nelson (1971), Reimchen (1983), Reimchen et al. (1985)). Boulton Lake *Gasterosteus* are polymorphic for the presence or absence of pelvic spines, ventral plate, and ascending process; we have restricted the morphological analyses to fish with full pelvic girdle and spines. Log-transformed morphometric data were analyzed with discriminant functions (BMDP 7M, Jenrich and Sampson 1983).

Results and discussion

Comparisons of the population centroids derived from the morphological measurements (Table 1) indicate highly significant differences between all pairwise combinations ($P < 0.001$), with Drizzle Outlet and Delkatla Estuary being most similar and Drizzle Lake and Boulton Lake being most dissimilar. Boulton Lake and Drizzle Lake stickleback are approximately equidistant in discriminant space from the Delkatla Estuary fish (Fig. 2). Inclusion of meristic data accentuates the separation between the populations since the Boulton Lake population is unique in having spine reduction, including frequent loss of dorsal and pelvic spines. Delkatla Estuary fish are further separated by the presence of 26 bony lateral plates rather than 3–5, as observed in the remaining populations.

The size of *G. aculeatus* mtDNA, 16.6 kilobase pairs (kb), was estimated by averaging the sums of the mtDNA fragment lengths from the *Eco*RI and *Hin*dIII digests and is well within the range (15.7–19.5 kb) found among most vertebrate mtDNAs (Brown 1985; Attardi 1985). Large mtDNA duplications were observed in one individual from Boulton Lake (1.1 kb) and in one from Delkatla Estuary (5.0 kb). Although duplications of this size have been found in several reptiles (Moritz and Brown 1986, 1987) and an amphibian (Wallis 1987), this is the first report of their occurrence in teleosts. The only other known mtDNA duplications in teleosts have been found in the brook stickleback, *Culaea inconstans* (M. H. Gach, unpublished data), and *Notropis* (T. Dowling, personal communication). mtDNA size variation of 70–180 base pairs was common among individuals in all populations. Intraspecific size polymorphism, once considered rare, is being documented with increasing frequency, particularly among lower vertebrates (Wright et al. 1983; Monnerot et al. 1984; Bermingham et al. 1986). Similar mtDNA size variation also occurs in the brook stickleback (M. H. Gach, unpublished data) and may be a common feature of the Gasterosteidae.

Seven mtDNA clones were revealed by restriction endonuclease digests. The three six-base restriction enzymes, *Ava*I, *Eco*RI, and *Hin*dIII, produced a total of 15 fragments corresponding to 14 restriction sites; the four-base enzymes, *Hin*fI and *Mbo*I, together yielded 60 fragments corresponding to 57 sites. The number of sites is less than the number of fragments because the gain of a restriction site results in the appearance of two novel fragments while a site loss produces a single novel fragment. The 71 sites sampled in this study represent a total of 312 nucleotide bases, or 1.9% of the *G. aculeatus* mitochondrial genome. Sequence divergence (Avise et al. 1979) among the seven clones was less than 1.0% in all cases, and represents a limited number of restriction site changes. For example, the two most divergent (0.7%) clones, clones 4 and 7, are interderivable by four substitution events (Fig. 3). Multiple clones were observed in every population but Drizzle Lake (Table 2). The number of clones relative to sample size did not differ significantly between populations (Fisher's exact test, $P < 0.05$). Frequencies of individual clones were, however, significantly different between certain populations. Freshwater population frequencies were independent of the Delkatla Estuary population, either singly

TABLE 1. Population comparisons of morphological and mtDNA characteristics

	Delkatla Estuary	Drizzle Outlet	Drizzle Lake	Boulton Lake
Delkatla Estuary	—	0.005	0.003	<0.001
Drizzle Outlet	45.7	—	0.333	0.098
Drizzle Lake	238.0	125.4	—	0.272
Boulton Lake	279.7	216.7	286.9	—

NOTE: Above diagonal, probability values (Fisher's exact test) of between-population mtDNA genotypic frequency comparisons; below diagonal, *F*-ratios of morphologic population means (discriminant function analyses, df = 5,100), restricted to adult male stickleback; $P < 0.001$ for all pairwise morphological comparisons.

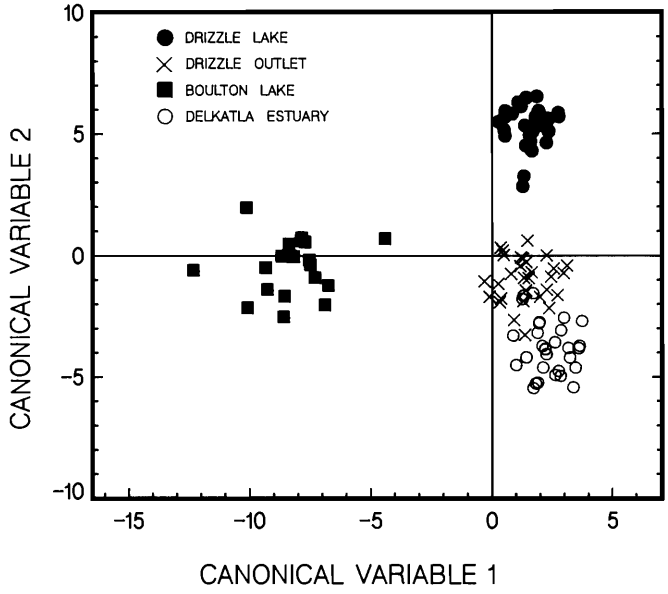

FIG. 2. Scatterplot on first and second canonical axes of morphometric measurements from four populations of *Gasterosteus aculeatus* from the Queen Charlotte Islands. Each point represents a single individual.

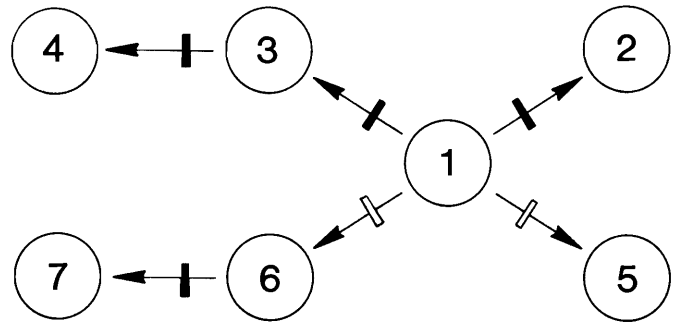

FIG. 3. Minimum length network of *G. aculeatus* mtDNA clones constructed using restriction site data. Clones are defined in Table 2. Directions of site changes have been determined relative to clone 1 and are not meant to convey direction of evolution. Solid vertical bars symbolize single restriction site gains, open bars are site losses.

TABLE 2. Results of *G. aculeatus* mtDNA endonuclease survey

Clone	Genotype*	Locality	Sample size	No. of restriction sites assayed
1	AAA	Delkatla Estuary	3	67
		Drizzle Outlet	1	
2	AAD	Delkatla Estuary	3	66
3	ABA	Drizzle Outlet	4	66
		Drizzle Lake	4	
		Boulton Lake	8	
4	ABB	Boulton Lake	4	65
5	ACA	Delkatla Estuary	1	66
6	BAA	Drizzle Outlet	1	66
7	BAC	Delkatla Estuary	1	65

*First letter (A or B), restriction patterns of *Eco*RI; second letter (A–C), restriction patterns of *Hin*fI; third letter (A–D), restriction patterns of *Mbo*I.

(Table 1) or in combination ($P < 0.001$). This is principally due to a *Hin*fI restriction site occurring in 20 of 22 freshwater individuals, but absent in all 7 estuarine fish (Table 2). Of the three freshwater populations, Drizzle Outlet was most similar to Delkatla Estuary in clone frequency ($P = 0.005$). Among the three freshwater populations, Drizzle Lake did not differ significantly in clonal composition from Boulton Lake or Drizzle Outlet, nor did Boulton Lake differ ($P = 0.098$) from Drizzle Outlet.

There is only limited association between morphological and mtDNA differentiation among these four populations (Table 1). Delkatla Estuary fish, which by one hypothesis may be the putative ancestor of the freshwater populations, are most similar to the Drizzle Outlet fish in both morphology and mtDNA, and are dissimilar in both morphology and mtDNA from fish of Boulton and Drizzle lakes. Among the freshwater populations, Boulton Lake and Drizzle Lake are the most morphologically divergent in discriminant space, to a degree comparable to the maximum divergence with the estuarine form; they appear, however, to have virtually identical mtDNA sequences.

The potential for gene flow differs substantially between these populations. Drizzle Lake is fully connected to Drizzle Outlet and thus to marine waters; hence movement of fish can potentially occur. Morphological assessment yielded no evidence for hybridization (Reimchen et al. 1985; present study), yet Drizzle Outlet fish contain clones found in both the lake (clone 3) and marine (clone 1) populations. Gene flow thus provides a tenable explanation for the clonal similarity between Drizzle Lake and Drizzle Outlet, and between the Drizzle Outlet and Delkatla Estuary populations. In contrast, stickleback from Boulton Lake are geographically isolated from all other populations. The lake does not have any inflow streams, but is maintained with groundwater seepage. Stickleback are absent from the outflow drainage because of its steep gradient (T. E. Reimchen, personal observation) and it is probable that this highly derived population has not been in contact with any other stickleback following its initial access to the lake. Consequently, any similarity in restriction sites between Boulton Lake fish and the remaining populations is probably not the consequence of gene flow.

Quaternary biotic refugia have been postulated for the Queen Charlotte Islands on the basis of endemism in a freshwater crustacean (Bousfield 1958), eight subspecies of birds and mammals (Foster 1965), angiosperms (Calder and Taylor 1968), bryophytes (Schofield 1969), and carabid beetles (Kavanaugh 1980). The Boulton Lake and Drizzle Lake *Gasterosteus* represent two opposite extremes of morphological divergence in freshwater populations (Moodie and

Reimchen 1976*b*) and would therefore be plausible candidates for relict populations. If these populations had been separated from each other since the maximum advance of the Late Wisconsin glaciation 18 000 years BP (Flint 1971), and assuming that mutation rates in *Gasterosteus* are comparable to those calculated for primates (0.02 base substitutions / 10^6 years; Brown et al. 1979), in sampling 2% of a 16.6-kb molecule we would expect to see 6 substitution site differences. Our mtDNA data, however, indicate virtually identical restriction patterns for these two isolated populations (a single *Mbo*I site difference in 4 of 12 Boulton Lake individuals; Table 2), consistent with a more recent origin of the morphological differences between these populations. These lakes are Holocene in origin, being underlain by Wisconsin glacial outwash gravels (Sutherland-Brown 1968). Radiocarbon dates of basal core sections taken at Boulton Lake and at Serendipity Pond, 16 km northeast of Drizzle Lake, both yield maximum ages of 8000 to 10 000 years (Warner 1984). The limited mtDNA divergence is consistent with this length of time for the geographical separation of the populations and supports suggestions (Moodie and Reimchen 1976*a*; Reimchen et al. 1985) that these endemic populations are examples of rapid postglacial morphological evolution.

It has been postulated that marine *Gasterosteus* are the source populations for the recolonization of watersheds following deglaciation and that freshwater populations from different watersheds are independently derived from the marine form (Wootton 1976; Bell 1976; Withler and McPhail 1985). This hypothesis predicts that mtDNA patterns should be more similar between marine and freshwater populations than between freshwater populations in different watersheds. Our data suggest that this may not be true for at least two of the populations in the Queen Charlotte Islands. Boulton Lake and Drizzle Lake stickleback are in drainages that discharge 50 km apart into marine waters. Consequently, these two *Gasterosteus* populations would be assumed to be independently derived from marine populations (Moodie and Reimchen 1976*a*). However, each individual sampled from these two lakes has a restriction site common to both populations yet absent in the estuarine fish. While insufficient sampling may account for the absence of this clone in the latter population, it is also conceivable that the two endemic populations (Boulton and Drizzle lakes) have diverged, instead, from a common freshwater ancestor. Periglacial ponds and streams in this area could have provided a refugium for such a source population. The possibility of such features is illustrated by the presence of a small coastal area 20 km to the east of Boulton Lake which remained ice-free during the height of the Wisconsin glacial advance (Warner et al. 1982). Since most of the northeastern region of Graham Island, on which the divergent populations are found, is a low-elevation (< 100 m) plain underlain by outwash gravels, early postglacial drainage patterns across this region may have been extensively dendritic, allowing access by this source population to many watersheds that are currently isolated from each other. Boulton Lake and Drizzle Lake have similar elevations (59 and 52 m, respectively) and are connected by low-elevation corridors (<61-m contour line). If this freshwater origin hypothesis is correct, the unique *Hin*fI restriction site should be found in many of the endemic populations from the region. In addition, nucleotide sequence divergence between anadromous and freshwater forms should be more pronounced in this region than in coastal watersheds where no refugia were present. Clearly, analysis of much larger samples is required to characterize the populations fully. A comparison with mainland coastal freshwater populations and their putative marine ancestors would be relevant in evaluating our conclusions.

Acknowledgements

We wish to thank T. Dowling and P. O'Reilly for discussion and comments on the manuscript and S. D. Douglas, J. D. McPhail, and G. R. Smith for aid in transporting fish. This work was facilitated by National Science Foundation grant BSR8516645 to the Laboratory of Molecular Systematics, University of Michigan Museum of Zoology, and by a Natural Sciences and Engineering Research Council of Canada grant to T.E.R.

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Some observations on *Pseudomenopon pilosum* (Amblycera: Menoponidae), the louse vector of *Pelecitus fulicaeatrae* (Nematoda: Filarioidea) of coots, *Fulica americana* (Aves: Gruiformes)

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Received April 18, 1988

BARTLETT, C. M., and ANDERSON, R. C. 1989. Some observations on *Pseudomenopon pilosum* (Amblycera: Menoponidae), the louse vector of *Pelecitus fulicaeatrae* (Nematoda: Filarioidea) of coots, *Fulica americana* (Aves: Gruiformes). Can. J. Zool. **67**: 1328–1331.

Breeding populations of *Pseudomenopon pilosum* (Scopoli, 1763) became established on 10 laboratory-reared juvenile American Coots (*Fulica americana* Gmelin) initially infested with 5 adult male and 5 adult female lice. Eggs of *P. pilosum* hatched less than 10 days after deposition and the combined duration of the three nymphal instars was 10–20 days. Nymphs and adults occupied all regions of the body. *Pseudomenopon pilosum* might thus acquire microfilariae of *Pelecitus fulicaeatrae* (Diesing, 1861) by simply randomly moving to and feeding on the legs where, in infected coots, the skin-inhabiting microfilariae of *P. fulicaeatrae* are known mainly to occur. *Pseudomenopon pilosum* occurred on all of 13 adult coots and three 1-week-old coot chicks collected in June in western Canada where *P. fulicaeatrae* is enzootic. Third-stage larvae of *P. fulicaeatrae* were found in adult *P. pilosum* on two of four adult coots harbouring microfilariae, but prevalence in lice was low (5.5% of 18 lice on one coot and 1.1% of 90 on the second) and only one third-stage larva was present in each infected louse. Four other species of lice were present on adult coots but only one other on 1-week-old chicks. Experiments showed that *P. pilosum* could occur as a straggler on chickens (*Gallus gallus* (L.)) and Red-necked Grebes (*Podiceps grisegena* (Boddaert)) although it did not establish on either species.

Printed in Canada / Imprimé au Canada