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ORIGINAL ARTICLE

# The distribution of divergent mitochondrial DNA lineages of threespine stickleback (*Gasterosteus aculeatus*) in the northeastern Pacific Basin: post-glacial dispersal and lake accessibility

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## ABSTRACT

**Aim** This study furthers the documentation of the geographical distribution of two divergent (*c.* 3%) mitochondrial DNA clades in the threespine stickleback (*Gasterosteus aculeatus*) and tests the hypotheses that the northeastern Pacific distribution has been influenced by post-glacial colonization and lake elevation and that clade identity is associated with certain morphological attributes such as reduction in body armour.

**Location** Lakes and nearshore marine environments of the eastern Pacific Basin from southcentral Alaska to southeastern British Columbia, (BC) Canada.

**Methods** Restriction enzyme analysis of polymerase chain reaction-amplified mitochondrial DNA fragments (cytochrome *b*) from a total of 45 new populations combined with existing data for a further 45 populations. Lake elevation data were collected for 78 localities and tested for an association with mtDNA clade by contingency table analyses. Morphological data were collected on sticklebacks from eight samples representing four lake-stream systems and tested for differentiation among populations with different mtDNA clade identities using analyses of variance.

**Results** We extend the known distribution of the haplotypes diagnostic of the Trans-North Pacific Clade (TNPC) southward to mid-Vancouver Island and, for the first time, on mainland BC, in other island populations far from putative refugia, and in nearby anadromous populations. A morphological analysis indicated that the mainland population with the TNPC was not characterized by reduced spine or lateral plate ('armour') traits that characterize some putative relict populations on the Queen Charlotte Islands. We found a significant association between lake elevation and the presence of the TNPC; the TNPC was present more often in lakes located at or lower than 42 m than in higher elevation lakes.

**Main conclusions** Our data support the hypothesis that post-glacial colonization by TNPC-bearing marine sticklebacks and aspects of lake 'accessibility' were important in determining the distribution of mtDNA clades in the eastern Pacific Ocean basin. More generally, our study demonstrates how processes acting both across immense geographic scales (e.g. pan-Pacific dispersal) and local scales (lake accessibility contingent on timing and extent of isostatic rebound) may interact to explain biogeographical patterns.

**Keywords**

**Lake elevation, mitochondrial DNA, Pacific Basin, phylogeography, post-glacial dispersal, threespine stickleback.**

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## INTRODUCTION

Determining the historical and contemporary forces that influence the distribution of a species or its traits is a central, but difficult question in biogeography especially because experimental evaluation of historical dispersal or vicariance is not feasible (Endler, 1982; Brown & Lomolino, 1998). It is possible, however, to indirectly evaluate historical causes of a biogeographical pattern by carefully analysing the distribution of variation in light of known ecological requirements and historical events (Brown & Lomolino, 1998; Mercer & Roth, 2003). The glacial cycles of the Pleistocene Epoch have influenced the distribution of many species worldwide (Brown & Lomolino, 1998; Hewitt, 2000). In particular, patterns of freshwater fish distributions on the once-glaciated northwest coast of North America have been attributed to glacial events (McPhail & Lindsay, 1986) including the distribution of morphological and genetic variation in the threespine stickleback, *Gasterosteus aculeatus* Linnaeus 1758 (Moodie & Reimchen, 1976a; McPhail, 1993; O'Reilly *et al.*, 1993; Taylor & McPhail, 2000). This freshwater and marine species shows remarkable variation throughout its circumboreal range, particularly in lakes on the coast of British Columbia (BC) (Moodie & Reimchen, 1976b; McPhail, 1994; Ortí *et al.*, 1994). Some variants, such as spine and lateral plate (i.e. 'armour traits') reduction, melanism and phylogenetic lineages are distributed discontinuously across regions (e.g. Haglund *et al.*, 1992; Lavin & McPhail, 1992; Ortí *et al.*, 1994).

For instance, *G. aculeatus* contains two ancient mtDNA lineages that differ by 2–3% in sequence (O'Reilly *et al.*, 1993; Ortí *et al.*, 1994; Thompson *et al.*, 1997). The Euro-North American clade (ENAC) is found in coastal lakes and marine waters throughout Europe and North America, including the west coast from Alaska to California (Ortí *et al.*, 1994). The Trans-North Pacific clade (TNPC, also known as 'Japanese' or 'Argonaut' clades; O'Reilly *et al.*, 1993; Thompson *et al.*, 1997) is found in freshwater and marine habitats near the seas of Japan and Okhotsk in the western Pacific, but it is rare relative to the ENAC in eastern Pacific waters. The TNPC may, however, reach locally high frequencies in some lakes and streams in the northeast Pacific basin (Deagle *et al.*, 1996; Thompson *et al.*, 1997). Despite extensive knowledge of the distribution of haplotypes that comprise the two major stickleback mitochondrial DNA lineages in BC and Alaska the origin of this distribution remains unexplained. For instance, certain lakes in BC and Alaska contain TNPC fish, but the TNPC haplotypes have not been found in lakes or streams south of northern Vancouver Island, BC (*c.* 52° N latitude; Ortí *et al.*, 1994; Deagle *et al.*, 1996; Thompson *et al.*, 1997; Taylor & McPhail, 1999; E.B. Taylor, unpublished data). Higuchi & Goto (1996) suggested that the TNPC originated approximately 2 Ma when lowered sea levels during the early Pleistocene isolated sticklebacks in the Sea of Japan from populations in the rest of the Pacific Ocean. The TNPC haplotypes may then have spread to the eastern Pacific using nearshore or offshore migration (Quinn & Light, 1989) and currently represent the surviving lineages from marine colonists from the western Pacific Ocean (Ortí *et al.*, 1994). Consequently, the presence of TNPC haplotypes in lakes north of a particular latitude (*c.* 52° N), but not further south, may represent the furthest extent of the TNPC's southward marine dispersal.

Alternatively, the scattered distribution of TNPC haplotypes in BC and Alaska may reflect its status as a freshwater 'relict' that colonized coastal lakes preglacially and persisted in refugia during the Pleistocene glaciations rather than reflecting post-glacial dispersal and colonization of freshwaters by marine stickleback (O'Reilly *et al.*, 1993). In support of this idea, the TNPC was initially found only in freshwater populations near a putative glacial refugium in and around the Queen Charlotte Islands (QCI) and northern Vancouver Island (the 'Hecate Strait Refugium', Clague, 1981; Warner *et al.*, 1982; Waitt & Thorson, 1983; Mann, 1986), but not in nearby marine populations (O'Reilly *et al.*, 1993; Ortí *et al.*, 1994; Deagle *et al.*, 1996; Thompson *et al.*, 1997). Further, Deagle *et al.* (1996) found an association between armour loss and presence of the TNPC in the QCI as further support for the relict hypothesis because this morphological differentiation suggested that the TNPC has had an extended (pre-glacial) freshwater history, similar to Japan Sea drainage freshwater sticklebacks (Higuchi & Goto, 1996). O'Reilly *et al.* (1993) acknowledged, however, that more extensive sampling of marine populations was needed, and that if the TNPC was found in nearshore marine populations it would refute the relict hypothesis as the sole source of TNPC sticklebacks. Indeed, TNPC sticklebacks were subsequently reported in a mid-Pacific Ocean sample suggesting ongoing dispersal across the Pacific. Haplotypes diagnostic of the TNPC have not, however, been found in marine samples around the QCI archipelago which would provide more compelling evidence of a post-glacial origin to TNPC sticklebacks in the eastern Pacific (Deagle *et al.*, 1996).

While marine post-glacial dispersal or the 'relict' hypothesis may explain broad-scale patterns in mtDNA clade distribution, it remains unclear why at smaller spatial scales and at latitudes where the TNPC is found, it is present in some lakes, but not others. One possibility is that postglacially dispersing sticklebacks bearing TNPC mtDNA were systematically restricted from certain lakes. The TNPC is largely absent from mountainous regions of the QCI (Deagle *et al.*, 1996), and TNPC-containing lakes on northern Vancouver Island are in lowland areas (Thompson *et al.*, 1997), suggesting that the North American distribution of the TNPC may be dependent on lake elevation or another factor limiting lake accessibility, an idea we designate the 'accessibility hypothesis'.

To further assess the geographical extent of post-glacial colonization of putative relict populations of TNPC sticklebacks, we sampled fish from a number of new localities in BC and Alaska, including four independent drainages on the central coast of BC, more extensive coverage of northern Vancouver Island, and several marine and anadromous populations. The areas of the BC coast sampled are further south and east than have been sampled to date and could help

to establish the extent of influence of the Hecate Strait Refugium on post-glacial dispersal of freshwater organisms, as well as the presence of the TNPC in marine sticklebacks. In addition, we characterized a total of 78 lakes in terms of their altitudes to test the null hypothesis that there is no association between mtDNA clade designation and lake accessibility to postglacially dispersing sticklebacks. Finally, we assessed armour traits in a subset of sticklebacks to see if an association between mtDNA clade designation and armour reduction reported by Deagle *et al.* (1996) is more widely applicable.

## MATERIALS AND METHODS

### Fish sampling

Stickleback from BC ($n = 24$ lake populations and 12 anadromous or marine populations) and Alaska ($n = 9$ lake populations) were collected using minnow traps and pole seines between 1995 and 2001 (Table 1; Fig. 1). A subset of populations were preserved in 4% formalin and stored in 37.5% isopropyl alcohol for morphological analysis. Individuals for genetic analysis were placed in 95% ethanol for DNA preservation. All fish sampled were anaesthetized with diluted MS-222 prior to preservation.

### MtDNA analysis

Ortí *et al.* (1994) identified several restriction enzyme sites within the cytochrome *b* gene that are diagnostic for *G. aculeatus* mitochondrial clades. We used two of these to identify haplotypes diagnostic of mtDNA lineage of central coast stickleback populations. DNA was first extracted from tissue using the Puregene® DNA Isolation Kit (Gentra Systems, Inc. Minneapolis, MN, USA), resuspended in 50 μL TE (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, both pH 8.0), or by Chelex extraction and stored at −20 °C. Tissue samples of approximately 0.5 g (wet wt) were taken from the tail fin of each adult individual; for juvenile (*c.* 20 mm) fish, the posterior third of the body was used. The polymerase chain reaction (PCR) was employed to amplify a segment of DNA specified by two oligonucleotide primers. The primers *GluD*-G and *Cyt*-B2 were used to amplify a 450 base pair segment of the mitochondrial cytochrome *b* gene (Palumbi, 1996). DNA was amplified in a total volume of 25 μL containing 0.6 μM of each primer, 0.8 μM dNTP mixture, 1.5 units of *Taq* DNA polymerase, 2 mM $MgCl_2$, 20 mM Tris HCl (pH 8.4), 50 mM KCl, and 2 μL of the DNA extraction product.

Amplifications were preformed in a PTC-100 Thermal Cycler (MJ Research, Waltman, MA, USA) using the following cycling conditions: a denaturation step at 95 °C for 3 min, followed by 1 min each of primer annealing at 52 °C and extension at 72 °C. Five similar cycles followed, but denaturation was at 94 °C. The next 31 cycles used 30 s each of 92, 55 and 72 °C, finishing with a final extension at 72 °C for 5 min. All PCR products were digested using the enzyme *Nsi*I. The presence of a *Nsi*I restriction site in the ENAC results in two fragments; the TNPC lacks a restriction site for this enzyme in the amplified sequence and produces only a single fragment after incubation (Ortí *et al.*, 1994). DNAs from fish previously assigned to either ENAC or TNPC were also digested as controls.

To increase the probability of correct TNPC lineage assignment, *Hin*c II was used in a second restriction digest for samples uncut by *Nsi*I. *Hin*c II has a restriction site within the amplified region in the TNPC, but not the ENAC (Deagle *et al.*, 1996). After enzyme incubation, fragments were separated by gel electrophoresis on 2% agarose, stained with ethidium bromide and visualized using ultraviolet light.

### Morphological measurements

To examine whether armour reduction is more frequently associated with populations containing the TNPC, the following measurements were made on eight samples (from four lake-stream systems): standard length, first dorsal spine length, left pelvic spine length, pelvic girdle length, and lateral plate number. Counts and measurements were made as described in Lavin & McPhail (1985), using either calipers (accurate to 0.1 mm) or the ocular micrometer of a dissecting microscope (at 6.4 × magnification). Prior to measuring, the fish were cleared using KOH and stained with Alizarin Red to allow more accurate measurement of armour characters and were transferred to 37.5% isopropyl alcohol for storage.

### Data analyses

To assess the elevation distribution of lakes containing TNPC stickleback, the current assays were combined with published *G. aculeatus* mtDNA assays (O'Reilly *et al.*, 1993; Ortí *et al.*, 1994; Deagle *et al.*, 1996; Thompson *et al.*, 1997). At least 17 other streams and rivers have been assayed (E.B. Taylor, unpublished data), but these were not included in our statistical analysis because (1) they could not be considered as data points independent of their adjacent lakes, (2) they spanned elevations and (3) data on their elevations were infrequently available. Elevation data were also not found for some of Deagle *et al.*'s (1996) sites because they lack gazetted names and exact locations were not given. Lakes were categorized as 'TNPC present' or 'TNPC absent'. To reduce the chance of incorrectly identifying a lake as 'TNPC absent', lakes without the TNPC clade were only included in our analyses if 10 or more individuals had been assayed ($n = 74$ lakes). A sample size of 10 is not ideal because it is unlikely that a low proportion of TNPC present in a lake would be detected (e.g. at least 20 fish are needed to detect the TNPC at a true frequency of 5%). Restricting the analysis to lakes with 20 fish analysed, however, would eliminate most available data (modal $n = 12$ fish). Sample sizes were also too small to accurately estimate the proportion of TNPC fish in a population as this may approach 0 or 1 (Zar, 1999). Consequently we restricted our analyses to presence/absence data.

**Table 1** Locations of threespine stickleback samples for mtDNA clade distribution

| Area | Name | Map code | *n* | Clade | Proportion TNPC | Elevation (m) | Source |
|---|---|---|---|---|---|---|---|
| AK | Corcoran Lake | 1 | 11 | 1 | 0.18 | 76.2 | 5 |
| AK | Stephan Lake | 2 | 27 | 0 | 0 | 30.5 | 5 |
| AK | Kalmback Lake | 3 | 21 | 0 | 0 | 106.7 | 5 |
| AK | Whale Lake | 4 | 9 | 0 | 0 | 61 | 5 |
| AK | Long Lake | 5 | 10 | 0 | 0 | 45.7 | 5 |
| AK | Matanuska Lake | 6 | 1 | 1 | - | 19 | 2 |
| AK | Prator Lake | 7 | 1 | 1 | - | 94 | 2 |
| AK | Mud Lake | 8 | 16 | 1 | 0.25 | 15.2 | 5 |
| AK | Tommelson Lake | 9 | 10 | 0 | 0 | 15.2 | 5 |
| QCI | Ain Lake | 10 | 12 | 0 | 0 | 46 | 2 |
| QCI | Anser Lake | 11 | 12 | 0 | 0 | 3 | 3 |
| QCI | Blowdown Lake | 12 | 12 | 0 | 0 | 42 | 3 |
| QCI | Boulton Lake | 13 | 23 | 0 | 0 | 57 | 1, 3 |
| QCI | Drizzle Lake | 14 | 13 | 0 | 0 | 52 | 1, 2 |
| QCI | Grus Lake | 15 | 12 | 0 | 0 | 4 | 3 |
| QCI | Hickey Lake | 16 | 12 | 0 | 0 | 32 | 3 |
| QCI | Juno Lake | 17 | 12 | 0 | 0 | 56 | 3 |
| QCI | Lower Victoria Lake | 18 | 10 | 0 | 0 | 40 | 3 |
| QCI | Mayer Lake | 19 | 15 | 0 | 0 | 23 | 1, 4 |
| QCI | Mesa Lake | 20 | 12 | 0 | 0 | 91 | 3 |
| QCI | Middle Lake | 21 | 12 | 0 | 0 | 91 | 3 |
| QCI | Parkes Lake | 22 | 12 | 0 | 0 | 73 | 3 |
| QCI | Pure Lake | 23 | 12 | 0 | 0 | 69 | 3 |
| QCI | Richter Lake | 24 | 12 | 0 | 0 | 70 | 1 |
| QCI | Skonun Lake | 25 | 12 | 0 | 0 | 50 | 3 |
| QCI | Solstice Lake | 26 | 12 | 0 | 0 | 69 | 3 |
| QCI | Slim Lake | 27 | 12 | 0 | 0 | 8 | 3 |
| QCI | Loon Lake | 28 | 12 | 0 | 0 | 15 | 3 |
| QCI | Wiggin Lake | 29 | 12 | 0 | 0 | 18 | 3 |
| QCI | Spraint Lake | 30 | 12 | 0 | 0 | 90 | 3 |
| QCI | Kumdis Pond | 31 | 12 | 0 | 0 | 0 | 3 |
| QCI | Mollitor Pond | 32 | 12 | 0 | 0 | 0 | 3 |
| QCI | N.Otter Lake | 33 | 12 | 0 | 0 | 20 | 3 |
| QCI | Red Truck Pond | 34 | 12 | 0 | 0 | 0 | 3 |
| QCI | Silver Lake | 35 | 12 | 0 | 0 | 0 | 3 |
| QCI | Midge Lake | 36 | 12 | 0 | 0 | 30.5 | 3 |
| QCI | Naked Lake | 37 | 12 | 0 | 0 | 30.5 | 3 |
| QCI | Pontoon Lake | 38 | 12 | 0 | 0 | 30.5 | 3 |
| QCI | Branta Lake | 39 | 12 | 0 | 0 | 61 | 3 |
| QCI | Stump Lake | 40 | 12 | 0 | 0 | 61 | 3 |
| QCI | Blue Danube Lake | 41 | 7 | 1 | 0.14 | 7 | 3 |
| QCI | Clearwater Lake | 42 | 41 | 1 | 0.54 | 21 | 3 |
| QCI | Escarpement Lake | 43 | 10 | 1 | 0.9 | 14 | 3 |
| QCI | Gudal Lake | 44 | 12 | 1 | 0.08 | 37 | 3 |
| QCI | Harelda Lake | 45 | 40 | 1 | 0.3 | 24 | 1, 3 |
| QCI | Imber Lake | 46 | 30 | 1 | 0.2 | 21 | 3 |
| QCI | Mica Lake | 47 | 12 | 1 | 0.08 | 9 | 3 |
| QCI | N. Lumme Lake | 48 | 12 | 1 | 0.83 | 8 | 3 |
| QCI | Chown Pond | 49 | 12 | 1 | 0.08 | 0 | 3 |
| QCI | Fife Swamp | 50 | 12 | 1 | 0.33 | 0 | 3 |
| QCI | Serendipity Lake | 51 | 67 | 1 | 0.46 | 20 | 3 |
| QCI | Sangan Pond | 52 | 12 | 1 | 0.08 | 30.5 | 3 |
| QCI | Rouge Lake | 53 | 12 | 1 | 1.00 | 30.5 | 3 |
| QCI | Skidegate Lake | 54 | 12 | 0 | 0 | 40 | 1 |
| CC | *Bella Coola River* | 55 | 12 | 0 | 0 | 0 | 5 |
| CC | *Princess Royal Island* | 56 | 15 | 0 | 0 | 0 | 5 |

**Table 1** *continued*

| Area | Name | Map code | *n* | Clade | Proportion TNPC | Elevation (m) | Source |
|---|---|---|---|---|---|---|---|
| CC | Jigsaw Lake | 57 | 20 | 1 | 0.40 | 15 | 5 |
| CC | Eva Lake | 58 | 12 | 0 | 0 | 20 | 5 |
| CC | Myra Lake | 59 | 10 | 0 | 0 | 20 | 5 |
| CC | Slingsby Lake | 60 | 10 | 0 | 0 | 20 | 5 |
| CC | *Seymour Inlet* | 61 | 10 | 0 | 0 | 0 | 5 |
| VI | William Lake | 62 | 19 | 0 | 0 | 120 | 5 |
| VI | *Goodspeed River* | 63 | 16 | 0 | 0 | 0 | 5 |
| VI | Quatse Lake | 64 | 27 | 0 | 0 | 76 | 5 |
| VI | Beaver Lake | 65 | 30 | 0 | 0 | 90 | 4 |
| VI | Misty Lake | 66 | 92 | 1 | 0.06 | 91 | 2, 4, 5 |
| VI | *Cluxewe River* | 67 | 34 | 1 | 0.17 | 0 | 5 |
| VI | *Nimpkish River* | 68 | 20 | 1 | 0.05 | 0 | 5 |
| VI | Nimpkish Lake | 69 | 16 | 0 | 0 | 20 | 5 |
| VI | Roselle Lake | 70 | 18 | 0 | 0 | 95 | 5 |
| VI | Anutz Lake | 71 | 10 | 0 | 0 | 30 | 5 |
| VI | Atluck Lake | 72 | 10 | 0 | 0 | 134 | 5 |
| VI | Woss Lake | 73 | 19 | 0 | 0 | 149 | 5 |
| VI | Vernon Lake | 74 | 20 | 0 | 0 | 220 | 5 |
| VI | *Kokish River* | 75 | 19 | 0 | 0 | 0 | 5 |
| VI | *Eve River* | 76 | 30 | 1 | 0.06 | 0 | 5 |
| VI | *Kelsey Bay* | 77 | 10 | 0 | 0 | 0 | 5 |
| VI | *Tidal Flats* | 78 | 30 | 0 | 0 | 0 | 5 |
| VI | *Little Bear Bay* | 79 | 22 | 1 | 0.05 | 0 | 5 |
| VI | McCreight Lake | 80 | 15 | 0 | 0 | 55 | 5 |
| VI | Mackie Lake | 81 | 30 | 0 | 0 | 150 | 5 |
| VI | Mud Lake | 82 | 16 | 0 | 0 | 213 | 5 |
| VI | Boot Lake | 83 | 12 | 0 | 0 | 240 | 5 |
| VI | Merril Lake | 84 | 12 | 0 | 0 | 260 | 5 |
| QI | Chonat Lake | 85 | 27 | 0 | 0 | 15 | 5 |
| QI | Village Bay Lake | 86 | 12 | 1 | 0.17 | 15 | 5 |
| QI | Main Lake | 87 | 16 | 1 | 0.063 | 23 | 5 |
| QI | Morte Lake | 88 | 20 | 1 | 0.95 | 105 | 5 |
| VI | *Little River* | 89 | 11 | 0 | 0 | 0 | 5 |
| VI | Sarita Lake | 90 | 12 | 0 | 0 | 55 | 5 |

Map code refers to locality codes shown in Fig. 1, and *n* represents sample size. Proportion Trans-North Pacific Clade (TNPC) was calculated for sample sizes of at least 5.

CC, British Columbia Central Coast; AK, Alaska; QCI, Queen Charlotte Islands; VI, Vancouver Island; QI, Quadra Island.

For 'Clade': 0, TNPC not detected; 1, TNPC detected. Anadromous or marine populations are in italics.

Sources: 1, O'Reilly *et al.* (1993); 2, Ortí *et al.* (1994); 3, Deagle *et al.* (1996); 4, Thompson *et al.* (1997); 5, current study.

Elevation data were obtained using FishWizard (http://pisces.env.gov.bc.ca/) an online source of BC lake information, and 1 : 50,000 topographical maps. For all Alaskan lakes and BC lakes not included on FishWizard, only approximate elevations (to the nearest 30.5 m or 100 ft) could be found. In order to include these lakes in statistical analyses, we arbitrarily assigned each the highest possible elevation (i.e. a lake between 0 and 100 ft on the map was assigned an elevation of 30.5 m). When we repeated all analyses with the *lowest* possible elevation (e.g. 0 m for the above example), the results did not change; therefore, we concluded that this small degree of uncertainty is not significant for our analyses. The mean elevation of all lakes was 42 m and we divided samples into those from 'low elevation' lakes (i.e. ≤42 m) and those from 'high elevation' lakes (>42 m). We tested the distribution of TNPC present and TNPC absent samples between low elevation and high elevation lakes using $\chi^2$ randomization tests (Roff & Bentzen, 1989). The elevation distributions of 'TNPC present' and 'TNPC absent' lakes were not normal, and could not be made so by square-root or natural log transformations (Zar, 1999). Therefore, we used a nonparametric Wilcoxon signed-ranks test (equivalent to Mann–Whitney test) to test for differences between the elevation distributions of these two lake classes (Zar, 1999). In addition, the mean

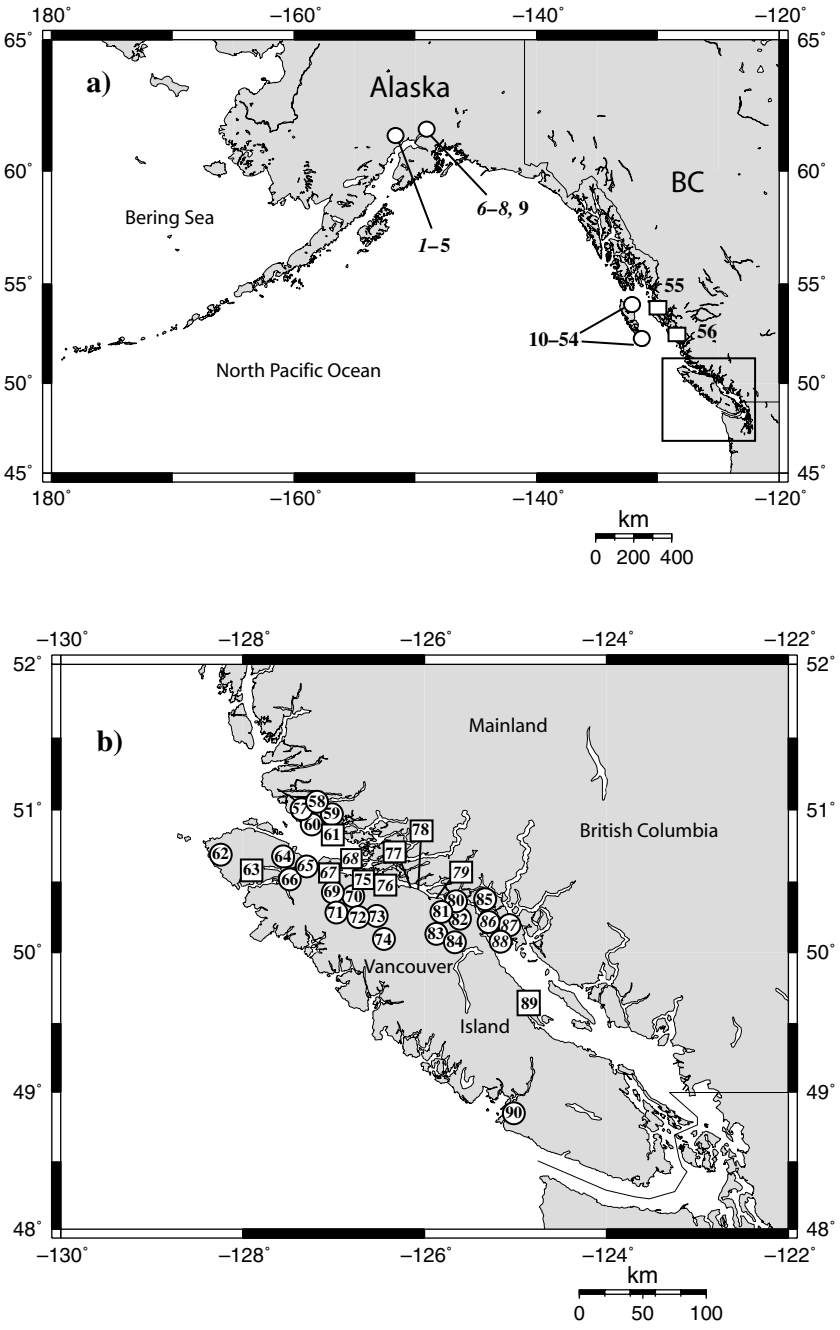


**Figure 1** Location of threespine stickleback populations characterized for mtDNA clade distribution in: (a) the North Pacific Ocean showing relative locations of Alaskan (1–9), Queen Charlotte Islands and adjacent mainland populations (10–56) and (b) the Vancouver Island area [boxed area in (a)]. Numbers designate population names in Table 1 and italicized numbers indicate lake (circles) and anadromous or estuarine populations (squares) in Alaska and British Columbia that contained Trans-North Pacific Clade mtDNA. Samples 10–54 represent previously published data (see O'Reilly *et al.*, 1993; Deagle *et al.*, 1996).

sample size for high elevation (>42 m) lakes was 18.1 fish (SE = 2.64) and that for low elevation lakes (≤42 m) was 16.2 fish which were not significantly different from one another (*t*-test, $P = 0.522$). The variable sample sizes among lakes, therefore, did not compromise the subsequent tests concerning lake elevation and, in fact, represent a conservative test of our hypothesis because the higher sample size in high elevation lakes makes it more likely to detect haplotypes from both mtDNA clades.

Analyses of variance (ANOVAS) were performed to determine if the sampled populations differed in any armour characters; when differences were found, multiple comparisons were made using Tukey's test (Zar, 1999). Plate counts did not meet the assumption of normality after square-root transformation. Therefore, the $\chi^2$ approximation of the Kruskal–Wallis test was used (Zar, 1999). The Bonferroni correction was used to allow multiple comparisons; significance levels for all four tests were thus set at $\alpha < 0.0125$ (Zar, 1999). The nonparametric equivalent of Tukey's test, which allows equal sample sizes, was used to compare mean values (Zar, 1999).

The residual comparison method was used to compare morphology in fish of different standard lengths (e.g. Vamosi *et al.*, 2000). For each variable (e.g. dorsal spine length), trait values for all measured fish were regressed against standard

length ($P < 0.05$ in all cases). Using the pooled, among-population regression equation for each trait, residuals (observed – value expected by equation) were calculated for each trait of each fish and these residuals were the basis of among population comparisons (Vamosi *et al.*, 2000). The Bonferroni correction was again employed, setting table-wide significance at $\alpha < 0.005$ ($k = 10$, Zar, 1999).

## RESULTS

The TNPC was identified in 12 of the 45 new localities included in our survey (Table 1). TNPC mtDNA was detected in four of the nine Cook Inlet (Alaska) lake populations, three of the four Quadra Island lake populations, one of 15 lake populations on northern Vancouver Island, one of the four adjacent mainland populations, and in four of the 12 anadromous or marine populations (Table 1).

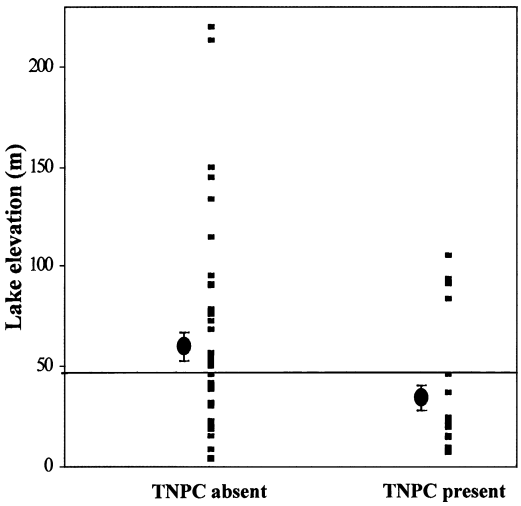


**Figure 2** Elevation distribution of the Trans-North Pacific Clade mtDNA in British Columbia and Alaska lakes. Dashes represent individual lakes, large circles represent mean elevation for each category ± standard error bars. The thick horizontal line represents the mean elevation of all lakes (42 m).

Over all lakes combined, the TNPC was present in proportionally more 'low elevation' lakes than 'high elevation' lakes ($\chi^2 = 9.3$, $P = 0.003$). The elevation distribution of 'TNPC present' lakes was significantly lower than that of 'TNPC absent' lakes, using the most conservative assignment of approximate elevations (Wilcoxon test using lower limit elevations; $P = 0.01$, Fig. 2). These general results appeared to be largely driven by the relationships within the more intensively sampled areas of the QCI and Vancouver Island and adjacent areas. In Alaska, for example, the mean elevations of TNPC-present lakes (51.1 m, $n = 4$) and TNPC-absent lakes (51.8 m, $n = 5$) were nearly identical. On the QCI and Vancouver Island, however, TNPC-present lakes were found at significantly lower elevations (17.1 m, $n = 13$ and 35.6 m, $n = 7$, respectively) than were TNPC-absent lakes (38.6 m, $n = 33$ and 104.3 m, $n = 19$, respectively, both $P < 0.025$).

The ANOVA and Kruskal–Wallis tests revealed differences between the eight measured populations in all armour characters (Table 2). For all traits, however, the population with the most TNPC fish (Jigsaw Lake) did not show the greatest reduction in armour traits. Eva Lake sticklebacks were the most armoured, followed by Jigsaw Lake fish. Myra and Slingsby populations were almost completely lacking in lateral plates and were reduced in other characters (Table 2).

## DISCUSSION

Our finding of the TNPC on the mainland of central coastal of BC, and south to Quadra Island, extends the known distribution of this clade to south and east of previously known locations centred on the QCI and northeastern Vancouver Island (O'Reilly *et al.*, 1993; Deagle *et al.*, 1996; Thompson *et al.*, 1997). Our data, therefore, are consistent with the possibility that the Hecate Strait Refugium extended into areas between northern Vancouver Island and the adjacent BC mainland (Waitt & Thorson, 1983). Deagle *et al.* (1996), however, first raised doubts about the universality of the relict hypothesis with their finding of TNPC sticklebacks on the QCI

**Table 2** Mean values for standard length and armour traits in eight samples of threespine stickleback sampled from four lake-stream systems

| Population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Trait | Myra L. | Myra Cr. | Eva L. | Eva Cr. | Slingsby L. | Slingsby Cr. | *Jigsaw L.* | *Jigsaw Cr.* |
| *n* | 30 | 10 | 30 | 8 | 30 | 12 | 30 | 15 |
| SL | 49.4 (0.72) | 49.8 (2.8) | 63.3 (2.5) | 58.1 (1.4) | 46.2 (0.46) | 53.0 (1.0) | 50.2 (0.77) | 52.7 (1.5) |
| DSL | 2.66 (0.06)$^a$ | 2.58 (0.11)$^a$ | 7.05 (0.36)$^b$ | 6.27 (0.12)$^b$ | 2.98 (0.06)$^c$ | 3.54 (0.15)$^c$ | 4.52 (0.11)$^d$ | 4.40 (0.09)$^d$ |
| PSL | 4.18 (0.11)$^a$ | 4.60 (0.25)$^a$ | 10.72 (0.53)$^b$ | 9.77 (0.13)$^b$ | 4.03 (0.09)$^a$ | 4.09 (0.69)$^a$ | 6.25 (0.12)$^c$ | 6.10 (0.09)$^c$ |
| PGL | 7.62 (0.14)$^a$ | 7.81 (0.17)$^a$ | 14.2 (0.78)$^b$ | 12.6 (0.36)$^b$ | 7.71 (0.14)$^c$ | 9.13 (0.73)$^c$ | 9.63 (0.20)$^a$ | 10.49 (0.30)$^a$ |
| LPN | 0.91 (0.25)$^a$ | 1.33 (0.66)$^a$ | 6.23 (0.45)$^b$ | 6.02 (0.09)$^b$ | 0.33 (0.11)$^a$ | 0.0 (0.0)$^a$ | 4.55 (0.12)$^c$ | 4.3 (0.13)$^c$ |

Mean values annotated with different letters are significantly different from one another (Tukey's test, $P < 0.05$). All measurements are in mm. Localities in italics contained TNPC mtDNA.

*n*, sample size; SL, standard length; DSL, dorsal spine length; PSL, pelvic spine length; PGL, pelvic girdle length; LPN, lateral plate number.

in areas far from putative refugia. These same authors, however, did not detect TNPC fish in estuarine samples of sticklebacks from the QCI, fish that would provide the most likely source for post-glacial colonists into lakes. We documented, however, the TNPC mtDNA in fish sampled from Quadra Island, which is much farther south and east than areas typically associated with the Hecate Strait Refugium. In addition, we found TNPC fish in anadromous and marine sticklebacks in coastal waters. These observations clearly suggest that the distribution of TNPC sticklebacks in BC was also influenced by post-glacial colonization of freshwater by marine sticklebacks (cf. Deagle *et al.*, 1996). Although, the TNPC was found in only 13 of 203 marine or anadromous fish (6.4%) sampled across 12 populations from mid-Vancouver Island to the Kitimat River on the BC Central Coast, postglacial colonization by small numbers of TNPC bearing marine sticklebacks and subsequent increase in TNPC haplotype frequencies by drift could easily explain the high frequencies of the TNPC haplotype in some lakes. In addition, the TNPC may have been more common in nearshore marine waters early in deglaciation than it is now. TNPC sticklebacks have also recently been found in anadromous populations in the Cook Inlet area, the same region as our Alaskan samples (M.A. Bell, G. Orti, T. Städler, L. Nevermann & A. Meyer, unpublished data, Suny, Stony Brook, NY, USA), which clearly establishes the presence of TNPC sticklebacks in marine populations across broad geographic range in the eastern Pacific Ocean (cf. Deagle *et al.*, 1996). These results are consistent with the hypothesis that TNPC sticklebacks in parts of Alaska, the QCI, and central BC have resulted from post-glacial colonization processes and that freshwater relict populations in areas of putative glacial refugia (O'Reilly *et al.*, 1993; Ortí *et al.*, 1994) are not necessary to explain the presence of TNPC sticklebacks in the eastern Pacific (although they may have also contributed post-glacial colonists).

Our analysis also focused on what post-glacial factors may have influenced the pattern of colonization of sticklebacks from distinct clades. We found that the TNPC was more frequently found in lower elevation lakes over its BC and Alaskan distribution, and that there was no association between the TNPC and armour-reduced populations. Given the glacial history of the BC coastline and its associated sea-level changes, the altitudinal distribution of lakes containing TNPC-bearing stickleback (Table 1, Fig. 2) also makes the relict hypothesis unlikely as a complete explanation for the broad distribution of mtDNA clades. At the end of the last glaciation, the melting of glacial ice caused eustatic sea level rises in excess of 100 m (Mann, 1986). Until isostatic rebound and tectonic uplift caused land to re-emerge, low coastal areas that are presently above sea level and contain lakes were submerged under marine waters. In fact, this process of submergence and rebound is one mechanism proposed for freshwater colonization by *G. aculeatus* and other euryhaline fishes from the marine environment (McPhail & Lindsay, 1986; McPhail, 1993). The exact altitude of post-glacial sea level rise varies by area and depends on the degree of local isostatic depression and rate of tectonic uplift. Post-glacial sea level rises (all relative to present) have been estimated at 100–120 m for the Central Coast study area (Clague, 1981), 92–200 m for northeastern Vancouver Island (Clague, 1981; Howes, 1982), 20–30 m for the QCI and (Clague *et al.*, 1982), and at least 86 m for the Cook Inlet area of Alaska (Reger & Pinney, 1996). Wave-eroded terraces and marine shell deposits at 200 m above present sea level on northern Vancouver Island confirm that sea level rises did occur, and radiocarbon dating of shell deposits to between 12,000 and 13,000 years before present confirm the timing of these rises (Clague, 1981). Using these estimates, all lakes on the central coast and northern Vancouver Island known to contain the TNPC presently were submerged. Similarly, most lakes on QCI were submerged except Gudal (37 m, contains the TNPC) and Skidegate (40 m, no TNPC; Table 1). Further, Rouge Lake was fixed for the TNPC, but the origin of this QCI lake has been dated to post-glacial time, between 5000 and 9000 years ago (Warner, 1984; Deagle *et al.*, 1996). In sum, under the relict hypothesis it is unclear how TNPC stickleback could have dispersed from a freshwater refugium to their present locations, survived post-glacial submergence, but remain largely restricted to low elevation lakes.

The observed pattern of reduced presence of TNPC haplotypes in high elevation lakes as well as our observation of the TNPC fish in nearshore marine and estuarine sticklebacks (Fig. 2, Table 1) suggests an additional factor that can explain the distribution of the TNPC in BC and Alaska. It is unknown when the once-isolated TNPC haplotypes first arrived in northeastern Pacific populations of marine stickleback. Fossil evidence, however, shows that sticklebacks were present in the eastern Pacific by at least 11 Ma (Bell, 1994). Consequently, if haplotypes of the TNPC did originate in the western Pacific approximately 2 Ma (Higuchi & Goto, 1996), it seems likely that ENAC haplotypes were already present in the central eastern Pacific when those of the TNPC arrived. If so, and TNPC haplotypes arrived at some intermediate point in the isostatic rebound process (when sea level dropped relative to land) then it would have been prevented from colonizing many high elevation lakes. High elevation lakes during this time would have been occupied by ENAC-bearing fish from the eastern Pacific that colonized these lakes before isostatic rebound. Under this scenario, low elevation lakes may contain haplotypes of both clades, or just one because of lineage extinction over time or perhaps even exclusion of haplotypes from one clade owing to prior residence by those of the other (Avise *et al.*, 1984; Deagle *et al.*, 1996; Hewitt, 1996). With the exception of Misty Lake at 91 m (Table 1; Fig. 2), our data are well explained by this hypothesis. The Misty Lake system, however, is part of the large, low gradient Keogh River that could have provided a relatively easy colonization route to Misty Lake, which is currently accessible to anadromous fishes (Irvine & Ward, 1989). It is also possible that recent colonization of high or low elevation lakes by TNPC-bearing sticklebacks could be prevented if their outlet streams are steeper, more distant from the ocean, or have more dispersal

barriers. For instance, there are waterfalls in the outlet streams of Myra and Slingsby lakes (this study) which are both below 20 m in elevation. Along with lineage extinction (Avise *et al.*, 1984), the presence of such barriers could explain the absence of TNPC haplotypes from many low elevation lakes (Fig. 2). Although we are still lacking important information such as the steepness and obstacles of outlet streams for each assayed lake population, the accessibility hypothesis appears to be an additional factor to explain the distribution of the TNPC on the coast of BC. Our observation that high elevation appeared most strongly associated with the lack of the TNPC in the QCI and Vancouver Island lakes, where we had samples from many lakes above the estimated maximal post-glacial sea level rises, is also consistent with a role for lake elevation. By contrast, we sampled only nine lakes in Alaska, but only two of those (one with the TNPC present) were located above the estimated 86 m post-glacial sea level rise, thereby limiting our ability to detect a relationship between elevation and clade distribution in Alaska.

The TNPC-bearing sticklebacks in drainages of the seas of Okhotsk and Japan have less extensive armour than their ENAC counterparts (Higuchi & Goto, 1996) and some BC populations with the TNPC also show greater armour reduction and support their status as pre-glacial relicts (O'Reilly *et al.*, 1993; Deagle *et al.*, 1996). Our morphological analysis, although limited in scope, provides no support for this association between mtDNA clade and armour phenotype and a similar situation has been reported for samples collected from Cook Inlet, Alaska (Bell, 1995; M.A. Bell, unpublished data). The lack of an association between armour traits and mtDNA lineage when samples over broad geographical areas are examined is probably a result of the greater variation in selective environments across the range of Pacific sticklebacks and the strong association between selective environments and armour traits. The armour traits measured – and many others in the threespine stickleback – have been shown to be subject to strong natural selection (Moodie, 1972; Bell, 1976; Reimchen, 1994; Schluter, 1994). An association between spine reduction and the absence of predatory fishes such as coastal cutthroat trout [*Oncorhynchus clarki clarki* (Richardson 1836)] and sculpins (*Cottus* sp. Linnaeus 1758) has long been known (reviewed in Bell, 1976). Gape-limited predators like cutthroat trout take longer to handle a well-armoured stickleback, often allowing the prey to escape (Moodie, 1972; Reimchen, 1994). Correlations between armour reduction and other environmental factors such as calcium limitation, which would make armour production difficult and costly, have also been demonstrated (Giles, 1983; Bell *et al.*, 1993). Recurrent evolution of reduced armour phenotypes across the distribution of *G. aculeatus* clearly demonstrates that these biotic and abiotic factors are likely more important than mtDNA or nDNA lineage in determining armour phenotypes (Bell *et al.*, 1993; Deagle *et al.*, 1996).

In conclusion, the results of our study suggest that post-glacial colonization of marine sticklebacks bearing TNPC mtDNA was an important process helping to explain variation in the distribution of the TNPC in BC and Alaska. Although our morphological analyses was limited to only four drainages and, therefore, constitutes a weak test, our data also suggest that there is no association between mtDNA lineage and armour reduction and does not support the hypothesis that this morphology marks relict populations (cf. Deagle *et al.*, 1996). Although the Hectate Strait Refugium probably contributed to biogeographical patterns in many taxa (e.g. see Byun *et al.*, 1999; McCusker *et al.*, 2000), including TNPC distribution, we suggest that colonization by marine sticklebacks, lake elevation and other correlates of accessibility are post-glacial factors that have had significant impacts on the current distribution of TNPC mtDNA in drainages tributary to the eastern Pacific Ocean. The distribution of mtDNA clades in *G. aculeatus* may, therefore, represent an additional example of the importance of historical contingency in explaining patterns of divergence in this species complex (cf. Taylor & McPhail, 2000). More generally, our study demonstrates how processes acting both across immense geographical scales (e.g. pan-Pacific dispersal) and local scales (lake accessibility contingent on timing and extent of isostatic rebound) may interact to explain biogeographical patterns.

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## BIOSKETCHES

**Lisa S. Johnson** completed a BSc Honour's degree at the University of British Columbia in 2002 studying stickleback phylogeography. She completed the field work for this research while working for Coastal Ecosystems. She has now entered a graduate journalism program at the University of British Columbia with a special interest in science journalism.

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