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GLOBAL SURVEY OF MITOCHONDRIAL DNA SEQUENCES IN THE THREESPINE STICKLEBACK: EVIDENCE FOR RECENT MIGRATIONS

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Abstract.—Phylogenetic analyses of mitochondrial DNA (mtDNA) sequences were used to assess the matriarchal genetic structure of the threespine stickleback, *Gasterosteus aculeatus*. A 747 base-pair (bp) fragment of the cytochrome *b* was sequenced from 36 individuals collected from 25 localities in Europe, North America, and Japan. Two major divergent clades were revealed: one widespread in Japan but with representatives in some Alaskan and British Columbian lakes and the other common in Europe and North America. A simple diagnostic test using the polymerase chain reaction (PCR) and a restriction enzyme was used to assay additional individuals, confirming the absence of the Japanese clade in the Atlantic basin. Geographic distribution of mtDNA variation suggests (1) a recent origin of the Atlantic populations, and (2) support for previous hypotheses about the existence of Pleistocene refugia for freshwater fishes in Alaska and British Columbia. Silent substitution rates were used to date the colonization of the Atlantic at 90,000 to 260,000 yr before present, which conflicts with earlier dates implied by the fossil record. The recent replacement of Atlantic mitochondrial lineages suggested by our data may be explained by severe reduction or extinction of northern Atlantic populations during the Pleistocene, followed by a recent reinvasion from the Pacific. With a global perspective of the distribution of genetic variation as a framework, meaningful comparisons at a smaller geographical scale will now be possible.

Key words.—Biogeography, cytochrome *b*, *Gasterosteus aculeatus*, mitochondrial DNA sequences, molecular phylogeny, Pleistocene refugia, polymerase chain reaction, substitution rate.

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Intraspecific studies of genetic variation based on mitochondrial DNA (mtDNA) have opened up a phylogenetic perspective on population biology (Wilson et al. 1985; Avise et al. 1987; Moritz et al. 1987; Avise 1989). The relative ease of obtaining mtDNA sequences by using the polymerase chain reaction (PCR) and direct sequencing (Kocher et al. 1989) allows for high-resolution analyses of population processes, as well as the study of molecular substitution patterns (reviewed in Meyer 1993a). Recent work has shown the utility of this approach for a variety of population-level questions in several species of fishes (e.g., Bartlett and Davidson 1991; Carr and Marshall 1991; Finnerty and Block 1992; Sturmbauer and Meyer 1992). A phylogenetic approach to the study of standing variation allows for the important distinction between historical and contemporary gene flow (Slatkin 1987). Studies of phenotypic evolution such as in morphological characters and allozyme frequencies usually lack this phylogenetic perspective (but see, e.g., Buth and Haglund 1994; Haglund et al. 1992, 1993).

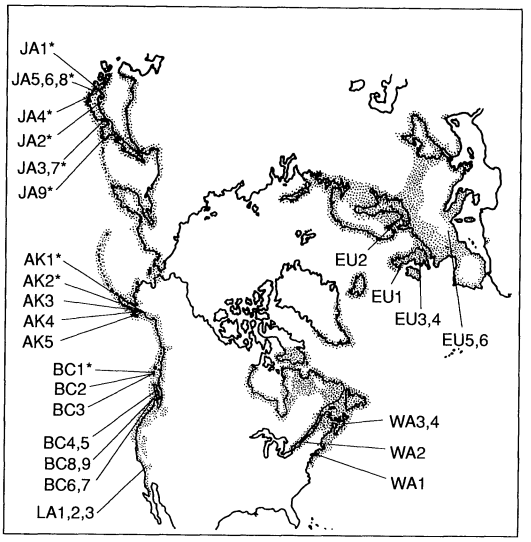
The threespine stickleback, *Gasterosteus aculeatus*, is a widespread circumboreal and north-temperate species mostly restricted to coastal regions (fig. 1). Three major life-history modes occur: fully marine, resident freshwater, and anadromous (entering freshwater only to breed). Freshwater populations are believed to have independently evolved from marine and anadromous ones (McPhail and Lindsey 1970; Bell 1976, 1984; Bell and Foster 1994a). Distinct morphologies associated with different freshwater habitats seem molded by selection and derived repeatedly under appropriate conditions (Hagen and Gilbertson 1972; Moodie 1972; Moodie and Reimchen 1976; Gross 1978; Reimchen 1980; reviewed in Bell 1976, 1984; Wootton 1976, 1984; Bell and Foster 1994a). Parallelism in morphological transformations is ubiquitous among freshwater geographic isolates of this species, hindering attempts to reconstruct historical relationships using phenotypic data. Given that subspecific recognition has been based on morphology (e.g., Miller and Hubbs 1969), considerable taxonomic confusion has ensued (Hubbs 1929; Penczak 1966; Wootton 1976; Bell 1984).

Pleistocene Glaciation greatly influenced pat-

terns of geographic variation in freshwater and coastal fishes of northern latitudes (Hocutt and Wiley 1986). As recently as 15,000 yr ago, vast areas were completely covered by the ice. Fishes now found in those regions must have immigrated since then either from the south or from ice-free refugia that have been postulated to have existed in Alaska and western Arctic Canada (McPhail and Lindsey 1970, 1986; O'Reilly et al. 1993). Morphology, fossils, and parasites have been used as evidence for suggesting dispersal routes and refugial origins of present-day faunas (Crossman and McAllister 1986), and differences among geographic forms have been attributed to divergence in isolated refugia during glaciation. For example, morphologically and genetically divergent freshwater populations of sticklebacks in the Queen Charlotte Islands have been suggested to have diverged in isolation in a Pleistocene refugium (O'Reilly et al. 1993). However, some of these differences may precede the last ice sheet and reflect older vicariant events, as will be shown below for mtDNA. Alternatively, geographic variants could have arisen rapidly after deglaciation. Postglacial morphological differentiation of freshwater sticklebacks now inhabiting several isolated drainages in Alaska might illustrate such cases (Bell et al. 1985; Francis et al. 1986; Bell and Ortí 1994). A more extensive understanding of the historical origins of patterns of variation observed in areas affected by glaciation should involve a global study of population structure covering the whole range of a species.

A recent specieswide allozyme survey assessed relationships among populations of *G. aculeatus* (Haglund et al. 1992). It revealed "very great" intraspecific genetic variation and two major genetic groups: a divergent set of populations from Japan versus all other populations. The former was suggested to become taxonomically distinguished from the rest of *G. aculeatus*. The latter were seen as a cohesive unit, albeit divided into two geographical subgroups: an Atlantic basin subgroup and an eastern Pacific subgroup which, interestingly, also included two populations from Japan. Congruence between this allozyme study and the present study will be discussed.

Here we present the results of a global survey of three-spine sticklebacks based on mtDNA sequences. Our approach allows for a detailed ex-



PAN, Port Ryotsu, Sado Island (marine, Y. Honma collector); JA2: JAPAN, Kanazawa, Ishikawa (marine, from D. Buth); JA3, 7: JAPAN, Maegata, Shariki, Aomori (marine, from D. Buth); JA4: JAPAN, Ohgaki, Gifu (freshwater, from D. Buth); JA5, 6: JAPAN, Biwako (Biwa Lake), Bunka (freshwater, M. Matsuoka collector); JA8: JAPAN, Biwa Lake, Shiga, Shiga (freshwater, from D. Buth); JA9: JAPAN, Ohnuma, Nanae, Hokkaido Island (marine, from D. Buth); AK1: ALASKA, Cook Inlet, Matanuska Lake (freshwater, M. Bell collector); AK2: ALASKA, Cook Inlet, Prator Lake (freshwater, M. Bell collector); AK3: ALASKA, Cook Inlet, Lampert Lake (freshwater, M. Bell collector); AK4: ALASKA, Deep Creek, Cook Inlet (anadromous, M. Bell collector); AK5: ALASKA, Cook Inlet, Headquarters Lake (freshwater, M. Bell collector); BC1: BRITISH COLUMBIA, Canada, Rouge Lake, Queen Charlotte Island (freshwater, T. Reimchen collector); BC2: BRITISH COLUMBIA, Canada, Delkatla Estuary, Queen Charlotte Island (anadromous; T. Reimchen collector); BC3: BRITISH COLUMBIA, Canada, Drizzle Lake, Queen Charlotte Island (freshwater, T. Reimchen collector); BC4, 5: BRITISH COLUMBIA, Canada, Paxton Lake (freshwater, D. Schluter and D. McPhail collectors); BC6, 7: BRITISH COLUMBIA, Canada, Misty Lake (freshwater, D. Schluter and D. McPhail collectors); BC8, 9: BRITISH COLUMBIA, Canada, Little Campbell River (marine, D. Schluter and D. McPhail collectors); LA1, 2, 3: LOS ANGELES, California, Ventura River (freshwater, M. Bell collector); WA1: NEW YORK, Flax Pond, Long Island (marine, M. Bell collector); WA2: QUEBEC, Canada, Isle Verte (anadromous, G. Rico collector); WA3, 4: NOVA SCOTIA, Canada, New Harbor River ("White stickleback," marine, D. Blouw collector); EU1: SCOTLAND, Sound of Mull, Argyllshire (anadromous, N. Campbell collector); EU2: SWEDEN, Fishebackskill (marine, A. Berglund collector); EU3, 4: ENGLAND, Pond in Leicester (freshwater, P. Taberlet collector); EU5, 6: FRANCE, Thonon-Les-Bains, Leman Lake (freshwater, P. Taberlet collector). Asterisks indicate samples that belong to the "Japanese clade" (see text and fig. 3).

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FIG. 1. Geographic distribution of *Gasterosteus aculeatus* (stippled areas) and sample localities. JA1: JA-

amination of intraspecific phylogeny based on gene genealogies, and provides a global perspective to previous observations from more restricted geographical areas. In particular, the magnitude and pattern of differentiation among mtDNA variants and their geographic distribution are discussed in terms of historical gene flow during the Pleistocene.

Materials and Methods

Specimens of *Gasterosteus aculeatus* that were used are listed in the legend of figure 1 with their localities, life-history strategies, and the source of the sample. Samples labeled JA2, JA3, JA4, JA7, JA8, and JA9 belong to populations sampled in the allozyme study of Haglund et al. (1992). British Columbia samples BC1, BC2, and BC3 from the Queen Charlotte Islands are from the same populations as those used by O'Reilly et al. (1993) and will allow comparison with results from their restriction-sites analysis. Alaskan samples derive from a survey of morphological differentiation among freshwater isolates (Bell and Ortí 1994). Specimens of the other species, used as outgroups, are *Gasterosteus wheatlandi* (Goose Neck Cove, Rhode Island; W. Krueger, Martin, and Sparsis collectors), and *Pungitius pungitius* (Freshwater Loch, Isle of Mubb, Scotland; R. Campbell collector). All specimens were preserved in 70% ethanol.

Total genomic DNA was extracted from white muscle or liver tissue by Proteinase K/SDS dissolution and purified by phenol-chloroform extraction and ethanol precipitation (Maniatis et al. 1982; Kocher et al. 1989). The polymerase chain reaction (PCR) (Saiki et al. 1988) was used to amplify a segment of the cytochrome *b* mitochondrial gene. Double-stranded amplifications were performed in 25 μL volumes containing 67 mM Tris (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM 2-mercaptoethanol, 1 mM of each dNTP, 1 μM of each primer, 10–1000 ng genomic DNA, and 0.5 units of Taq Polymerase (Cetus). Primers used were L14724 (5′-CGAAGCTTGATATGAAAAACCATC-GTTG-3′; located in the glutamine tRNA, Pääbo 1990; Meyer et al. 1990), and H15525 (5′-TTT-GCAGGGGTAAAATTATCAGGAT-3′) to obtain an amplification product 831 base pairs (bp) long. Gel purification (2.5% Nusieve-Agarose in TAE buffer) of the double-stranded product was followed by generation of single-stranded DNA for direct sequencing (Gyllensten and Erlich 1988), using asymmetric polymerase chain reaction (PCR) with L14724 as limiting primer. Single-stranded DNA was concentrated and desalted in spin columns (Millipore: Ultrafree-MC30,000) and sequenced with L147424 and L15018 (5′-GCYAAYGGCGCATCCTTYT-TYTT-3′, where Y = C or T) by the dideoxy method using a commercial kit (Sequenase Version 2, United States Biochemical).

The orthologous DNA sequences obtained were aligned by eye with a multiple sequence editor (ESEE, Cabot and Beckenbach 1989). Intraspecific variation in *G. aculeatus* was quantified by computing haplotype diversity (*h*) and nucleotide diversity values (Π) according to Nei and Tajima (1981) and Nei (1987). Phylogenetic analyses were based on three different methods. Parsimony analyses were performed using PAUP (Swofford 1991), maximum-likelihood (Felsenstein 1981), and neighbor-joining (Saitou and Nei 1987) analyses using the PHYLIP package (Felsenstein 1991). Bootstrapping (Felsenstein 1985) was used with all three methods to estimate statistical confidence in the data. Mean number of substitutions per silent site (K_s) between sequences were estimated according to the method of Pamilo and Bianchi (1993) and Li (1993), with the computer program LI93 kindly provided by W.-H Li.

Nineteen additional samples were extracted and amplified as above but cut with a restriction enzyme (*Bst* XI) diagnostic for the major clades found (see Results). A 10 μL aliquot of the double stranded PCR amplification product was mixed with 10 μL of a solution containing 200 mM NaCl, 33 mM Tris-Cl pH 7, 13 mM $MgCl_2$, and 4 units of *Bst* XI (Boehringer Mannheim), and incubated for 2 h at 45°C. The digested product was resolved on a 2% agarose minigel and visualized by ethidium bromide staining. All sticklebacks assayed by this method were from the Atlantic basin, as follows: two individuals from Flax Pond, Lond Island, New York (marine, M. A. Bell collector); two individuals from Goose Neck Cove, NewPort, Rhode Island (marine, W. Krueger collector); three individuals from Middle Medford, Nova Scotia, Canada (anadromous, D. Blouw collector); four individuals from Isle Verte, Quebec, Canada (anadromous, G. Rico collector); three individuals from the North Sea, Scotland (marine, N. Campbell collector); three individuals from Fishebackskill, Sweden (marine, A. Berglund collector); two individuals from Lehman Lake, Thonon-Les-Bains, France (freshwater, P. Taberlet collector).

Results

We obtained 747 base pairs (bp) of DNA sequence from the 5′ end of cytochrome *b* from 36 individuals of *Gasterosteus aculeatus* and the two outgroups (fig. 2). Average sequence divergence (uncorrected) between *G. aculeatus* and the outgroups was 10.3% (from *Gasterosteus wheatlandi*) and 14.2% (from *Pungitius pungitius*), and it was 15.8% between the outgroups. The mean number of substitutions at silent sites between *Pungitius* and *Gasterosteus* was estimated as 0.7407–0.8481.

Substantial intraspecific variation was observed among *G. aculeatus*. A total of 35 nucleotide positions varied (table 1), 29 of which were third codon positions, and six were first positions; no variation in second positions was found. This variation defined 17 distinct haplotypes (A–Q, table 1) among all fish assayed. The most divergent haplotypes (C and Q) differed by 23 substitutions (3.08%), 20 of which were transitions and three of which were transversions, with only two inferred amino-acid replacements. Silent substitution differences ranged from 0.0748 to 0.1007 for haplotypes G–O and B–P, respectively. Only four conservative amino-acid replacements (valine for isoleucine or phenylalanine) were inferred from the DNA sequences (fig. 2). Based on the cladogram presented in figure 3, a total of 34 transitions and five transversions are needed to derive the 17 haplotypes from their common ancestor.

Diversity values broken down by geographic region are summarized in table 2. Considered altogether, haplotypic and nucleotide diversities in the threespine stickleback were 0.935 and 0.007, respectively. Thirteen haplotypes were found among the Pacific basin samples, and only 4 in the Atlantic. As a consequence, haplotypic diversity is significantly higher ($t = 2.552$, $P < 0.02$) in the Pacific than in the Atlantic. Likewise, nucleotide diversity is eight times larger in the Pacific than in the Atlantic. A comparison of Japanese ($N = 9$) versus European ($N = 6$) samples, which cover comparable geographic areas, shows a significantly higher ($t = 3.527$, $P < 0.01$) haplotypic diversity in Japan (table 2).

The 17 haplotypes can be divided easily into two major clades (A–H and I–Q), which differ by at least 18 substitutions. Figure 3 shows a consensus tree for the stickleback haplotypes generated by PAUP. A branch-and-bound search (all characters unordered, equal weights) generated seven shortest trees (length = 192 steps, CI excluding uninformative sites = 0.80, rescaled consistency index = 0.87). Monophyly of *G. aculeatus* is well supported (bootstrap value of 100%). The two major lineages within the threespine stickleback are defined by nine synapomorphies each and high bootstrap values (99%). However, groupings within these lineages are not well sustained, with the exception of a European clade (haplotypes P and Q) that has a strongly suggestive bootstrap value of 84%. Neighbor-joining and maximum-likelihood analyses produced congruent topologies and similar bootstrapping results.

A striking pattern is revealed when geographic locality is superimposed on the cladogram (fig. 3). The two major lineages do not correspond to Atlantic versus Pacific: both of them occur in the Pacific. They correspond roughly to Japanese versus Euro-North American, but members of the two clades are geographically interspersed in Alaska and British Columbia. The highest local nucleotide diversity values are found in this area (table 2), reflecting the presence of haplotypes from both clades. In contrast, very low diversity was found among all haplotypes of Atlantic origin, which form a group within the Euro-American clade. Haplotype M from Los Angeles groups with Atlantic haplotypes (minimum difference of two substitutions), rather than with other eastern Pacific haplotypes (minimum difference of five substitutions), but this grouping is not well supported by bootstrap analysis.

To assay additional fish to determine to which major clade they belong, a simple restriction enzyme test was developed. A diagnostic site was found at position 255 (see table 1, fig. 3) for *Bst* XI after searching all 17 cytochrome *b* haplotype sequences with the computer program MACVECTOR (International Biotechnologies, Inc.). A thymine in this position (synapomorphic for the Euro-American clade) determines a restriction site for this enzyme, not present in any of the Japanese haplotype sequences. Therefore, digestion of the 831-bp PCR product with *Bst* XI yields two fragments (length 527 bp and 304 bp) for the Euro-American clade, and a single (uncut) fragment of 831 bp for the Japanese clade (fig. 4). All additional 19 fish from the Atlantic basin assayed with *Bst* XI were identified as belonging to the Euro-American clade. Other enzymes (not used in this study) having diagnostic restriction sites present in the Euro-American clade but absent from the Japanese clade se-

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Ppu  ATG GCA AGC CTA CGA AAA ACG CAC CCT CTA CTA AAA ATC GCT AAC AAT GCA TTA GTT GAC 60
Gwh  ATG GCA AGC CTA CGA AAA ACG CAC CCC CTA CTA AAA ATC GCA AAC AAC GCA CTA GTT GAT
Gac  ATG GCA AGC CTA CGA AAA ACG CAC CCC CTA CTA AAA ATY GCT AAC AAT GCA CTA RTC GAC
AA     M   A   S   L   R   K   T   H   P   L   L   K   I   A   N   N   A   L  V/I  D

Ppu  CTC CCC GCC CCC TCA AAT ATC TCC GTA TGG TGA AAC TTT GGT TCC CTA CTT GGA CTC TGC 120
Gwh  CTC CCA GCC CCT TCA AAT ATT TCG GTA TGA TGA AAC TTT GGT TCC CTA CTT GGA CTT TGC
Gac  CTC CCM GCC CCC TCA AAT ATT TCA GTR TGA TGA AAC TTT GGT TCC CTC CTT GGA CTT TGC
AA     L   P   A   P   S   N   I   S   V   W   W   N   F   G   S   L   L   G   L   C

Ppu  TTA ATT ATC CAA ATC CTA ACT GGC CTT TTT CTT GCT ATA CAT TAC ACA TCA GAC ATT GCC 180
Gwh  TTA ATT ATC CAA ATT CTT ACC GGG CTT TTC CTT GCA ATA CAT TAT ACT TCT GAT ATT GCT
Gac  TTA ATT ATC CAA WTT CTC ACT GGG CTT TTC CTT GCA ATR CAC TAC ACT TCC GAT ATT GCT
AA     L   I   I   Q  F/I  L   T   G   L   F   L   A   M   H   Y   T   S   D   I   A

Ppu  GCA ACA TTT TCA TCC GTC GGA CAT ATT TGC CGA GAC GTA AAC TAC GGA TGG CTA ATC CGA 240
Gwh  ACA GCT TTT TCT TCT GTT GGG CAC ATC TGT CGA GAT GTA AAC TAC GGC TGA CTA ATC CGA
Gac  ACA GCT TTT TCC TCC RTC GGA CAY ATC TGC CGA GAT GTR AAT TAC GGY TGA CTA ATT CGA
AA     T   A   F   S   S  V/I  G   H   I   C   R   D   V   N   Y   G   W   L   I   R

Ppu  AAC CTA CAT GCC AAT GGA GCA TCA TTT TTC TTC ATC TGT ATT TAC ATA CAC ATC GGA CGA 300
Gwh  AAC CTC CAC GCC AAC GGC GCA TCC TTT TTC TTT ATC TGT ATC TAC ATG CAC ATC GGC CGA
Gac  AAC CTC CAY GCC AAY GGC GCA TCC TTT TTC TTC ATC TGT ATC TAT ATR CAT ATY GGC CGA
AA     N   L   H   A   N   G   A   S   F   F   F   I   C   I   Y   M   H   I   G   R

Ppu  GGA CTT TAC TAT GGC TCC TAC TTA TAC AAA GAA ACA TGA AAC ATT GGG GTT GTT CTA CTA 360
Gwh  GGA CTC TAC TAC GGC TCT TAC TTA TAT AAG GAG ACC TGA AAC ATT GGA GTG GTC TTT CTA
Gac  GGA CTT TAC TAT GGC TCT TAY YTA TAY AAA GAR ACC TGA AAC ATY GGA GTR RTT CTT CTA
AA     G   L   Y   Y   G   S   Y   L   Y   K   E   T   W   N   I   G   V  V/I  L   L

Ppu  CTT TTA GTC ATA ATA ACA GCA TTC GTT GGG TAC GTC CTA CCA TGG GGA CAA ATA TCC TTC 420
Gwh  CTT TTA GTT ATA ATA ACA GCT TTC GTT GGT TAT GTT CTT CCA TGA GGA CAA ATA TCT TTC
Gac  CTT TTA GTH ATR ATA ACA GCC TTY GTT GGT TAT GTC CTY CCA TGA GGA CAA ATR TCT TTC
AA     L   L   V   M   M   T   A   F   V   G   Y   V   L   P   W   G   Q   M   S   F

Ppu  TGG GGT GCC ACA GTC ATC ACC AAC CTT CTA TCA GCT GTC CCC TAT GTG GGC AAC TCG CTA 480
Gwh  TGA GGA GCC ACA GTT ATC ACT AAC CTA CTC TCA GCC GTT CCC TAC GTT GGC AAC TCC TTA
Gac  TGA GGA GCT ACR GTT ATT ACC AAC CTA CTT TCA GCC GTC CCA TAC RTT GGY AAY TCA TTA
AA     W   G   A   T   V   I   T   N   L   L   S   A   V   P   Y   V   G   N   S   L

Ppu  GTA CAA TGA ATC TGA GGC GGC TTT TCT GTC GAC AAC GCT ACC CTA ACC CGA TTC TTT GCC 540
Gwh  GTT CAA TGA ATT TGA GGA GGC TTT TCT GTT GAC AAC GCC ACT CTA ACC CGC TTC TTT GCC
Gac  GTT CAA TGA ATT TGA GGR GGC TTT TCC GTT GAC AAC GCC ACC TTA ACA CGT TTC TTT GCC
AA     V   Q   W   I   W   G   G   F   S   V   D   N   A   T   L   T   R   F   F   A

Ppu  TTC CAC TTT CTA TTT CCC TTT GTT ATT GCT GGG GCC ACA TTA GTT CAC CTT CTG TTC CTC 600
Gwh  TTC CAC TTC TTA TTT CCT TTC GTA ATT GCC GGT GCT ACA CTT GTT CAC CTT CTC TTC CTT
Gac  TTT CAC TTC TTA TTC CCA TTT GTS ATT GCT GGT GCC ACA CTT GTT CAC CTT CTT TTC CTT
AA     F   H   F   L   F   P   F   V   I   A   G   A   T   L   V   H   L   L   F   L

Ppu  CAC CAA ACC GGC TCA AAC AAC CCC CTC GGC CTA AAC TCA GAC GCT GAC AAA ATC TCG TTC 660
Gwh  CAC CAA ACA GGC TCG AAT AAT CCC CTT GGC CTC AAC TCA GAC GCT GAT AAA ATC TCT TTC
Gac  CAC CAR ACA GGC TCT AAT AAC CCY CTT GGC CTG AAC TCA GAC GCT GAC AAA ATC TCC TTT
AA     H   Q   T   G   S   N   N   P   L   G   L   N   S   D   A   D   K   I   S   F

Ppu  CAC CCA TAC TTT TCT TAT AAA GAC CTC CTT GGC TTT GCA GCC CTA CTT GTC TCC CTC ACA 720
Gwh  CAC CCT TAC TTT TCT TAT AAA GAC CTG CTT GGT TTC GCA GCC CTA CTT ATT GCC TTA ACA
Gac  CAC CCC TAC TTT TCT TAT AAA GAC CTC CTW GGC TTC GCA GCC CTA CTA ATT GCC CTT ACA
AA     H   P   Y   F   S   Y   K   D   L   L   G   F   A   A   L   L   I   A   L   T

Ppu  GCC CTG GCA CTC TTC GCC CCT AAT CTG 747
Gwh  TCA CTA GCA CTA TTT GCA CCA AAC CTG
Gac  TCC CTA GCA CTA TTT GCT CCT AAC CTG
AA     S   L   A   L   F   A   P   N   L
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quences are *Nsi* I (cuts when there is a guanine in position 288) and *Eag* I (cuts when there is a cytosine in position 294) (see table 2).

Discussion

Nucleotide Substitution Pattern

Because the mitochondrial cytochrome *b* gene has been used widely as a genetic marker, its evolutionary dynamics are relatively well known (Irwin et al. 1991; Esposti et al. 1993; Meyer 1993a,b). The pattern of nucleotide substitutions in *Gasterosteus aculeatus* agrees with that found in other fishes (e.g., Smith et al. 1989; Beckenbach et al. 1990; Meyer and Wilson 1990; Meyer et al. 1990; Bartlett and Davidson 1991; Carr and Marshall 1991; McVeigh et al. 1991; Finnerty and Block 1992) and vertebrates (Kocher et al. 1989; Irwin et al. 1991). The high transition/transversion ratio (7/1) and the preponderance of third position silent substitutions (83%) observed is typical for comparisons of closely related species and populations within species. Purine transitions and pyrimidine transitions were equally frequent among the 35 variable sites surveyed (16 pyrimidine and 15 purine transitions, table 1), in agreement with the pattern observed in cod (Carr and Marshall 1991) but not with the excess of pyrimidine transitions observed in blue marlin (Finnerty and Block 1992). The four amino-acid replacements found among stickleback haplotypes are conservative (Grantham 1974), as are those reported for intraspecific comparisons in cod and trout (Beckenbach et al. 1990; Carr and Marshall 1991). Overall, the maximum intraspecific differentiation (3%) found between stickleback cytochrome *b* sequences exceeds previously published values for the same gene in cod, Atlantic salmon, blue marlin, four species of tuna, and several cichlid fishes (Meyer et al. 1990; Bartlett and Davidson 1991; Carr and Marshall 1991; McVeigh et al. 1991; Finnerty and Block 1992; Sturmbauer and Meyer 1992). Compared with restriction-fragment-based values summarized by Avise (1989), the observed high level of intraspecific variation in *G. aculeatus* suggests an old age for the vicariant event separating the major clades.

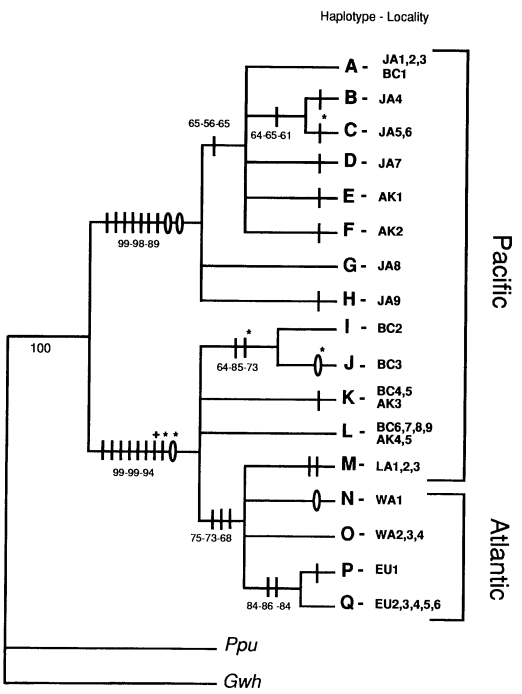

FIG. 3. Majority rule bootstrap consensus tree summarizing geographic locations and relationships among 36 individuals of *Gasterosteus aculeatus* (haplotypes A–Q) and two outgroups. Lines across branches indicate transition substitutions and ovals transversions, as reconstructed by PAUP (ACCTRAN option). Numbers are bootstrap values computed by 1000 replications in PAUP, followed by bootstrap values obtained with 100 replications with the NEIGHBOR program using maximum-likelihood distances, and by values using DNAML (PHYLIP, Felsenstein 1991). Asterisks indicate amino-acid replacements. The synapomorphic *Bst* XI site for the Euro-American clade is indicated by a cross.

Estimates of sequence divergence based on restriction enzymes and on DNA sequences may differ because of several factors (e.g., Wilson et al. 1985; Thomas and Beckenbach 1989; Taberlet et al. 1992; Meyer 1993a,b). A comparison of our results with those of a concurrent analysis using 10 restriction endonucleases (O'Reilly et al. 1993) is possible because both studies surveyed the same populations in the Queen Charlotte Islands, British Columbia. O'Reilly et al.

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FIG. 2. Consensus sequence of 747 bp from the 5′ end of cytochrome *b* for 36 individuals of *Gasterosteus aculeatus* (*Gac*), a single individual of *Gasterosteus wheatlandi* (*Gwh*), and a single individual of *Pungitius pungitius* (*Ppu*). The 35 variable positions in the *G. aculeatus* sequences are shown bold and underlined (symbols follow IUB code: R = G/A; Y = C/T; W = A/T; M = A/C; S = C/G; H = A/C/T). The inferred amino-acid sequence of *G. aculeatus* is presented below its nucleotide sequence, and the variable sites are shown bold and underlined.

TABLE 1. Nucleotide composition at variable sites and geographic distribution of the 17 distinct mtDNA *Gasterosteus aculeatus* genotypes. JA, Japan; AK, Alaska (USA); BC, British Columbia (Canada); LA, Los Angeles (USA); WA, West Atlantic; EU, Europe. Asterisk shows the diagnostic restriction site recognized by *Bst*XI.

	Nucleotide position																															Distribution					
Geno-type	3 9	5 5	6 6	8 7	1 3	1 3	1 9	2 6	2 4	2 9	2 8	2 9	2 5	2 8	2 4	3 1	3 2	3 7	3 3	3 5	3 4	3 5	3 1	3 2	3 9	3 2	3 4	4 9	4 4	4 2	4 6	4 1	4 7	4 8	5 4	6 6	6 4	6 0	JA	AK	BC	LA	WA	EU
													*																									
A	C	G	A	A	A	G	T	A	T	C	C	A	T	C	T	C	G	C	A	G	T	G	C	T	G	A	G	C	T	A	G	A	C	A	3	.	1	.	.	.
B	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	1	.	.	.	.	.
C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	2	.	.	.	.	.
D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	1	.	.	.	.	.
E	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
H	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
I	.	.	C	G	.	.	.	C	.	C	T	T	G	C	.	C	.	A	T	.	.	C	.	.	.	A	.	.	.	C	G	C	G	T	T	.	.	1	.	.	.
J	.	.	C	G	T	.	.	C	.	C	T	T	G	C	.	C	.	A	T	.	.	C	.	.	.	A	.	.	.	C	G	C	G	T	T	.	.	1	.	.	.
K	.	.	C	.	.	.	.	C	G	C	T	T	G	C	.	C	.	A	T	.	A	C	.	.	.	A	.	.	.	C	G	C	G	T	T	.	1	2	.	.	.
L	.	.	C	.	.	.	.	C	.	C	T	T	G	C	.	C	.	A	T	.	A	C	.	.	.	A	.	.	.	C	G	C	G	T	T	.	2	4	.	.	.
M	.	.	C	.	.	.	.	.	.	.	T	T	G	C	T	C	.	A	T	.	A	C	A	.	C	A	.	.	.	C	G	C	G	T	T	.	.	.	3	.	.
N	.	.	C	.	.	.	.	.	.	.	T	T	G	C	.	C	.	A	T	.	A	A	A	.	.	A	.	.	.	C	G	C	G	T	T	.	.	.	.	1	.
O	.	.	C	.	.	.	.	.	.	.	T	T	G	C	.	C	.	A	T	.	A	C	A	.	.	A	.	.	.	C	G	C	G	T	T	.	.	.	.	3	.
P	.	.	C	.	.	.	.	.	.	.	T	T	G	C	.	C	.	A	T	G	A	C	A	T	.	A	G	.	.	C	G	C	G	T	T	.	.	.	.	.	1
Q	.	.	C	.	.	.	.	.	.	.	T	T	G	C	.	C	.	A	T	G	A	C	A	.	.	A	G	.	.	C	G	C	G	T	T	.	.	.	.	.	5

TABLE 2. Haplotype diversity (h) and nucleotide diversity (Π) values for *Gasterosteus aculeatus* samples grouped by locality, where N is sample size.

Locality*	N	Number of haplotypes	Haplotype diversity (h ± SD)	Nucleotide diversity (Π)
Japan	9	6	0.8889 ± 0.0553	0.0012
Alaska	5	4	0.9000 ± 0.0888	0.0068
B. Columbia	9	5	0.8056 ± 0.0774	0.0033
W. Atlantic	4	2	0.5000 ± 0.1687	0.0003
Europe	6	2	0.3333 ± 0.1475	0.0002
Pacific	26	13	0.9138 ± 0.0217	0.0075
Atlantic	10	4	0.7111 ± 0.0764	0.0009
Total	36	17	0.9349 ± 0.0130	0.0071

* The Los Angeles sample was not treated as a single locality because it included only three fish from a single watershed.

found a maximum divergence of 2.46% between Rouge Lake (BC1) and Drizzle Lake (BC3) haplotypes, very close to the 2.68% sequence divergence found for cytochrome *b* between BC1 and BC3. Likewise, similarly low divergence (1.15%) between north Pacific and Atlantic sticklebacks estimated with restriction-sites analyses agrees with the 0.4%–1.2% sequence difference found between haplotypes J–P and L–O, respectively. These results suggest that the restriction enzymes used did not disproportionately sample the rapidly evolving control region. In a similar comparison in birds, Taberlet et al. (1992) used 17 restriction enzymes and 903 bp of cytochrome *b* but found a somewhat larger discrepancy in divergence values (0.55%–0.66% based on DNA sequences versus 1.13% ± 0.53% based on restriction enzymes).

mtDNA and Electrophoretic Data

A global phylogenetic analysis of allozyme variation in threespine stickleback (Haglund et al. 1992) produced results roughly congruent with ours. That study recognized two primary clades within *G. aculeatus*: (1) a basal Japanese clade, and (2) a group consisting of North American, European, and some Japanese populations. A striking difference between these results and ours is the relation between eastern and western Pacific populations. Some Japanese populations showed allozyme characters that resembled North American stickleback populations, but populations resembling Japanese allozyme profiles were absent from North America. In contrast, samples from North America (Alaska and British Columbia) contained Japanese mtDNA haplotypes, but North American haplotypes were absent in Japan. Sampling may, in part, account for these differences. The nine Japanese sticklebacks (from six populations) used in this study were part of the samples used by Haglund et al. (1992). Although we assayed mtDNA from five Alaskan (n = 5) and three British Columbian (n = 9) populations, among which only three (AK1, AK2, and BC1) contained Japanese haplotypes, Haglund et al. (1992) surveyed only allozymes from one freshwater population in Alaska. This single population sampled could have been composed of sticklebacks from the Euro-American clade. Also, the discrepancy may be caused by the dif-

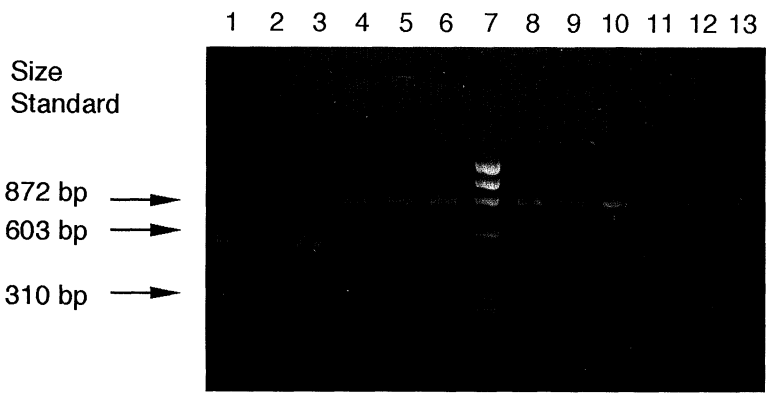

FIG. 4. Restriction fragment patterns for polymerase chain reaction (PCR) amplified DNA digested with *Bst* XI. Lanes 8–13 are undigested controls. Lane 7, ϕX174 RF DNA/*Hae* III size standard showing 1353bp, 1078bp, 872bp, 603bp, 310bp, 281bp, 234bp, 194bp, and 118bp fragments. Lanes 1–3 are Euro-American haplotypes (Q, M, and F, respectively) showing two restriction fragments (527bp and 304bp) and lanes 4–6 are Japanese haplotypes (G, F, and E, respectively) uncut by the enzyme.

ferent properties of allozymes and mitochondria or to differences in analytical approach. Phylogenetic treatment of electrophoretic data is not without problems (e.g., Buth 1984; Murphy et al. 1990). Only charge differences distinguish allozymes of a given locus, and electrophoretic mobility will often be a composite character rather than a discrete synapomorphy (Avise 1989). For intraspecific studies, recombination and natural selection can blur the historical record of allozyme markers. Karl and Avise (1992) have suggested that contradictory results obtained with allozyme markers and mtDNA in a geographic survey of oyster populations were likely caused by balancing selection acting on protein electrophoretic characters. They emphasized the need for caution in interpreting results that rely on assumptions of selective neutrality for allozyme markers. Mitochondrial DNA haplotypes differing by neutral substitutions, however, provide unambiguous information directly interpretable as gene genealogies. It should be noted, however, that sound inferences about organismal genealogy would be better if based on more than a single gene (Karl and Avise 1992).

Age of the Atlantic Basin Colonization

The significantly higher mtDNA diversity found in the Pacific, reflecting the presence of the two major clades, suggests that most of the evolutionary history of threespine sticklebacks occurred in this oceanic basin. The fossil record seems to support this notion, because *Gasterosteus* has been reported from Pacific basin deposits at least 11 my old (or possibly even 16 my old), whereas the oldest Atlantic basin fossil sticklebacks date back to only 1.9 my (Bell 1993). Atlantic mtDNA haplotypes of *G. aculeatus* form a derived clade within the Euro-American clade, closely related to eastern Pacific forms (fig. 3). All fish assayed with *Bst* XI were also assigned to the Euro-American clade, as did three fish examined by O'Reilly et al. (1993). A total of 32 Atlantic basin stickleback (from nine localities) surveyed by this and the previous study strongly suggests the absence of Japanese mtDNA haplotypes in this basin. This finding is also in agreement with allozyme data based on 240 individuals from six populations (Haglund et al. 1992). All Atlantic basin sticklebacks sequenced here contained only four distinct haplotypes, with maximum sequence divergence of 0.5%. The low level of genetic variation found among Atlantic samples (table 2) is consistent with a recent colonization but could also be a consequence of severe bottlenecks in Atlantic populations during Pleistocene glaciation. A similarly low pattern of genetic variation in Atlantic sticklebacks was also observed in the allozyme study of Haglund et al. (1992). These authors estimated genetic divergence between Atlantic North American and European populations (F_{ST} = 0.199) of the same magnitude as that found among samples from Pacific North America alone (F_{ST} = 0.163), and much smaller than Pacific North American plus some Japanese populations (F_{ST} = 0.408). Small allozyme variation was also reported among marine and freshwater populations of eastern Europe (Rafinski et al. 1989).

Interestingly, mtDNA lineage sorting between oceans is not complete (see fig. 3): the Los Angeles haplotype (M) is in the same clade with the Atlantic lineages (N–Q). Phylogenetic partitions are sometimes expected to lack concordance with population subdivision when the separation event is recent (Tajima 1983; Avise et al. 1984; Neigel and Avise 1986; Pamilo and Nei 1988; Avise and Ball 1990). In expanding populations, lineage extinction is dramatically slowed, therefore extending the time for lineage sorting further (Avise et al. 1984). Stickleback populations colonizing the Atlantic must have experienced pronounced range expansions, which might have favored the survival of ancestral Pacific mtDNA lineages. Recency of interoceanic common ancestry is strongly suggested by incomplete lineage sorting.

The time scale for the colonization of the Atlantic by Pacific sticklebacks may be estimated using molecular rates of evolution (e.g., Brown et al. 1979; Ferris et al. 1983; Wilson et al. 1985, 1987; DeSalle et al. 1987; Avise 1989; Avise et al. 1992). Although careful calibration for molecular rates of evolution within specific groups is desirable (e.g., Hillis and Moritz 1990), only a single significant date from the stickleback fossil record is available (Bell 1993). The oldest *Pungitius* fossils are from the Pacific (no fossil record exists for this genus in the Atlantic) and date back to 7 my, suggesting a minimum age of divergence from *Gasterosteus* of around 10 my (Bell 1994; see above). With this date and the estimated 0.74–0.85 divergence at silent sites between *Pungitius* and *G. aculeatus,* the silent divergence rate for cytochrome *b* in gasterosteid fishes is 7.4%–8.5% per million yr. This value is lower than the 10% rate estimated for cyto-

chrome *b* of mammals by Irwin et al. (1991), but higher than the 2.5% value for fourfold degenerate sites reported for bonnet-head sharks (Martin et al. 1992). Using this crude calibration, the 0.76%–1.91% silent divergence observed between Los Angeles and Atlantic sticklebacks would represent approximately 90,00 to 260,000 yr.

The Pacific stickleback lineage that colonized the Atlantic must have been dwelling in the north Pacific (close to the Bering Strait) at the time of colonization (assuming trans-Arctic migration). The Bering Seaway opened about 3.5 mya, allowing a great faunal interchange between the North Pacific and Arctic-Atlantic basins for a variety of marine organisms (Herman and Hopkins 1980; Vermeij 1991). A strong bias of movement in the direction of the Atlantic was reported based on recent and fossil geographic distributions (Durham and MacNeil 1967; Athaniasiadis 1990; Maggs 1990; Vermeij 1991). Dunton (1992) presents a brief description of the process by which unidirectional movement of marine organisms occurred. Our findings agree with this hypothesis about the direction of trans-Arctic migration, but the mtDNA data suggest that colonization of the Atlantic has been more recent. The fossil record of Atlantic sticklebacks from Plio-Pleistocene deposits (Bell 1994) dates the transition to the Atlantic about 2 mya, in contrast with the estimated 90,000 to 260,000 yr based on mtDNA. A possible explanation for this discrepancy could be an overestimation of the silent substitution rate for cytochrome *b,* because it is based on a minimum divergence date for *Gasterosteus* and *Pungitius* (see above). Older fossils of *Gasterosteus* and *Pungitius* would suggest a lower divergence rate for stickleback mtDNA, and thus an older colonization of the Atlantic. But to account for a 2-my-old presence of *Gasterosteus* in the Atlantic in terms of mtDNA divergence, it is necessary to postulate a silent divergence rate at least eightfold slower that the one estimated here. This higher rate, in turn, would push back the divergence between the two genera to the Cretaceous (80 mya), an unlikely event because the Gasterosteiformes are first known from the lower Eocene (Lauder and Liem 1983). We suggest that a recent replacement of mitochondrial lineages might have occurred in the Atlantic as a consequence of severe bottlenecks or even extinction of early northern Atlantic populations during the late Pleistocene, followed by reinvasion from the Pacific. Alternatively, early records of fossil *Gasterosteus* from Europe may have been misdated.

A similar pattern of disagreement between fossil evidence and mtDNA variation was observed in sea urchins from the northern Atlantic and Pacific (Palumbi and Kessing 1991). Extirpation of Atlantic populations was also postulated and attributed to harsher Plio-Pleistocene environmental fluctuations in the Atlantic than in the northwestern Pacific (cf. Palumbi and Kessing 1991; Vermeij 1989). Other marine species showing small interoceanic genetic distances include tuna, blue marlin, and green sea turtles, but for these eurythermic species, unlike sticklebacks, occasional contemporary gene flow through southern oceans is possible (Graves et al. 1984; Graves and Dizon 1989; Bowen et al. 1992; Finnerty and Block 1992). Large genetic distances observed between subspecies of cod and sister species of herring in different oceans (Grant and Utter 1984; Grant 1986; Grant and Ståhl 1988) were attributed to divergence since the opening of the Bering Strait (3–3.5 mya).

Pleistocene Refugia

Several Pleistocene ice-free refugia for freshwater and coastal fishes during the last (Wisconsin) glaciation have been postulated for the North Pacific region (McPhail and Lindsey 1970, 1986; Crossman and McAllister 1986; Lindsey and McPhail 1986). Particularly interesting is the suggestion that the climate probably was not much cooler than it is now in some parts of Alaska around Bristol Bay and Cook Inlet, where coastal and freshwater sticklebacks could have survived during the last ice age (McPhail and Lindsey 1970, 1986). Although geological evidence indicates effectively full glacial inundation of the Queen Charlotte Islands (British Columbia) during the Wisconsin (Blaise et al. 1990), stratigraphic evidence (Warner et al. 1982), globally disjunct plant distributions (Ogilvie 1989; Schofield 1989), and endemism in vertebrates (Foster 1965) and beetles (Kavanaugh 1980) cumulatively support the possibility of a Pleistocene glacial refugium in the vicinity of the Queen Charlotte Islands.

Therefore, the finding of a highly divergent mtDNA haplotype in Rouge Lake sticklebacks (in the Queen Charlotte Islands) led O'Reilly et al. (1993) to suggest a refugial origin for this freshwater population. With sampling localities restricted to British Columbia only, these authors found it difficult to explain the high mtDNA

divergence found between marine sticklebacks and this unique freshwater population, and postulated an extended preglacial history of isolation in freshwater habitats of the Queen Charlotte Islands during most of the Pleistocene. However, this same haplotype (A, sample BC1) is shown here to be widespread in Japanese marine populations (samples JA1–JA3) and to belong to a clade mostly restricted to Japan (figs. 1, 3). Interestingly, closely related Japanese mtDNA haplotypes were also found in Alaskan lakes north of Cook Inlet (samples AK1 and AK2 from the Mat-Su Valley), likewise close to a putative refugium. But populations with Japanese haplotypes were absent from lakes on the Kenai Peninsula (AK3–AK5) that is closer to the sea. The global perspective on mtDNA variation in *G. aculeatus* presented here (fig. 3) allows the distinction of two allopatric and highly divergent clades found to coexist only in freshwater habitats close to putative refugial areas in the northern Pacific. This pattern of phylogenetic discontinuity, not associated with spatial separation, has been attributed to secondary admixture zones (phylogeographic category II sensu Avise et al. 1987). On the basis of the silent divergence rate for cytochrome *b* (see above), these two clades are estimated to have diverged 0.9–1.3 mya, during the mid-Pleistocene and are not likely the product of vicariant events caused by the most recent glaciation. Blue tits exhibiting this phylogeographic pattern in the Grenoble region of France have been suggested to be postglacial colonizers coming from different refugia (Taberlet et al. 1992). Among threespine sticklebacks, a most significant aspect of this secondary contact area is the fact that the Japanese mtDNA haplotypes occur only in freshwater lakes, whereas marine sticklebacks always belong to the Euro-American clade. O'Reilly et al. (1993) surveyed mtDNA restriction profiles from 21 fish from marine localities from British Columbia, all of which can be safely assigned to our Euro-American clade (because the BC1–BC3 samples were shared by both studies). Therefore, this and the previous study failed to show the presence of Japanese mtDNA haplotypes among a total of 25 northern Pacific anadromous or marine sticklebacks assayed (four localities, from British Columbia to Alaska).

Sampling error could account for the absence of Japanese haplotypes in marine samples in the northern Pacific, *if* these haplotypes were currently present in low frequency or geographically restricted. However, the close affinity among Rouge Lake, Japanese, and Alaskan freshwater sticklebacks does suggest wide distribution of this clade in the Pacific. Genetic flow to the eastern Pacific from the west seems plausible by the occurrence of sticklebacks in the open North Pacific, some 900 km from land (Quinn and Light 1988). Furthermore, allozyme data demonstrate minimal population substructuring in marine habitats (Withler and McPhail 1985). Another alternative and more likely explanation is that this clade could have been abundant in the northern Pacific before the onset of the Wisconsin glaciation, and that advance of the Wisconsin ice sheet could account for its displacement out of the area, except from ice-free freshwater refugia near Cook Inlet, the Queen Charlotte Islands, and possibly elsewhere. A more extensive sampling of northern Pacific localities might confirm this hypothesis if Japanese haplotypes were found to occur only in freshwater localities close to putative refugia.

Systematics of the Gasterosteus aculeatus Superspecies

The use of subspecies for *G. aculeatus* based on morphological variation has generated much controversy and is considered at best dubious (see Wootton 1976). *Gasterosteus aculeatus* constitutes a cohesive taxonomic unit (Bell 1976), albeit with a division of standing mtDNA variation into two divergent clades. An ancestral vicariant event in the Pacific must have caused early divergence of these two clades. Subsequent range expansions and contractions of stickleback populations caused by successive advances and retreats of Pleistocene glaciations have originated secondary admixture zones in the northern Pacific. The presence of halotypes of both clades in some lakes of the Queen Charlotte Islands (O'Reilly et al. 1993) suggests that admixture of these two clades has been common, at least after the last glaciation. On the basis of their global allozyme survey, Haglund et al. (1992) advocated taxonomic recognition for a distinct subset of Japanese populations, forming the sister group of *G. aculeatus* Linneaus. This distinction is not warranted by the mtDNA phylogeny presented here, because all Japanese fish belong to the same clade.

Samples of an undescribed species of *Gasterosteus,* the "white stickleback," reproductively isolated from and sympatric with "typical" *G. aculeatus* in Nova Scotia (Blouw and Hagen 1984,

1990), were also included in our study (samples WA3 and WA4). Very small (0.1%) or no genetic divergence was detected between "white stickleback" and other western Atlantic sticklebacks, in agreement with a previous allozyme study (Haglund et al. 1990) and the postulated recency of the Atlantic lineage. A very recent origin of reproductive isolation between these forms is apparent.

With a global perspective of the distribution of genetic variation as a framework, meaningful comparisons at a smaller scale will now be possible. For example, it would be interesting to identify genetic affinities of morphologically derived populations in Alaska, and of sympatric species pairs of sticklebacks that coexist in several lakes in British Columbia (McPhail 1984, 1994; Schluter and McPhail 1992). Mitochondrial DNA could be used to test the hypothesis of repeated, consecutive colonizations from different marine haplotypes to lakes where sympatric species pairs occur (McPhail 1984). Haplotypes from both major clades were found to coexist in stickleback populations in lakes in the Queen Charlotte Islands (O'Reilly et al. 1993). Mitochondrial genes and morphology clearly evolve at different rates. Highly morphologically derived "giant stickleback" (sample BC3 from the Queen Charlotte Islands) are reproductively isolated from parapatric "typical" stream sticklebacks (Moodie 1972; Moodie and Reimchen 1976; Reimchen et al. 1985), but show very little mtDNA divergence (fig. 3, O'Reilly et al. 1993). We anticipate that mtDNA will be a useful marker to test hypotheses about speciation and isolating mechanisms, morphological divergence, and post-glacial distributions in this widely studied species.

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Literature Cited

Athaniasiadis, A. 1990. Evolutionary biogeography of the north Atlantic antithamnoid algae. Pp. 219–240 *in* D. J. Garbary and G. R. South, ed. Evolutionary biogeography of the marine algae of the North Atlantic. NATO ASI Series, Berlin.

Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43:1192–1208.

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Pp. 45–67 *in* D. Futuyma and J. Antonovics, eds. Oxford surveys in evolutionary biology. Oxford University Press, Oxford.

Avise, J. C., J. E. Neigel, and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. Journal of Molecular Evolution 20:99–105.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Avise, J. C., B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. Molecular Biology and Evolution 9: 457–473.

Bartlett, S. E., and W. S. Davidson. 1991. Identification of *Thunnus* Tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome *b* genes. Canadian Journal of Fisheries and Aquatic Sciences 48:309–317.

Beckenbach, A. T., W. K. Thomas, and H. Sohrabi. 1990. Intraspecific sequence variation in the mitochondrial genome of rainbow trout (*Oncorhynchus mykiss*). Genome 33:13–15.

Bell, M. A. 1976. The evolution of phenotypic diversity in *Gasterosteus aculeatus* superspecies on the Pacific coast of North America. Systematic Zoology 25:211–227.

———. 1984. Evolutionary phenetics and genetics: the threespine stickleback, *Gasterosteus aculeatus,* and related species. Pp. 431–528 *in* B. J. Turner, ed. Evolutionary genetics of fishes. Plenum, New York.

———. 1994. Paleobiology and evolution of threespine stickleback. Pp. 438–471 *in* Bell and Foster 1994b.

Bell, M. A., and S. A. Foster. 1994a. Introduction to the evolutionary biology of the threespine stickleback. Pp. 1–27 *in* Bell and Foster 1994b.

Bell, M. A., and S. A. Foster, eds. 1994b. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M. A., and G. Ortí. 1994. Pelvic reduction in threespine stickleback from Cook Inlet lakes: Geographical distribution and intrapopulation variation. Copeia 1994(2):314–325.

Bell, M. A., R. C. Francis, and A. C. Havens. 1985. Pelvic reduction and its directional asymmetry in threespine sticklebacks from the Cook Inlet Region, Alaska. Copeia 1985:437–444.

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

Blouw, D. M., and D. W. Hagen. 1984. The adaptive significance of dorsal spine variation in the four-spine stickleback, *Apeltes quadracus*. IV. Phenotypic covariation with closely related species. Heredity 53:383–396.

———. 1990. Breeding ecology and evidence of reproductive isolation of a widespread stickleback fish (Gasterosteidae) in Nova Scotia, Canada. Biological Journal of the Linnean Society 39:195–217.

Bowen, B. W., A. B. Meylan, J. Perran Ross, C. J. Limpus, G. H. Balazs, and J. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881.

Brown, W. M., M. J. George, and A. C. Wilson. 1979. Rapid evolution of mitochondrial DNA. Proceedings of the National Academy of Sciences, USA 76: 1967–1971.

Buth, D. G. 1984. The application of electrophoretic data in systematic studies. Annual Review of Ecology and Systematics 15:501–522.

Buth, D. G., and T. R. Haglund. 1994. Allozyme variation in the *Gasterosteus aculeatus* complex. Pp. 64–84 *in* Bell and Foster 1994b.

Cabot, E. L., and A. T. Beckenbach. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Computational Applied Bioscience 5:233–234.

Carr, S. M., and D. Marshall. 1991. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome *b* gene of Atlantic cod (*Gadus morhua*) detected by the polymerase chain reaction. Canadian Journal of Fisheries and Aquatic Sciences 48:48–52.

Crossman, E. J., and D. E. McAllister. 1986. Zoogeography of the freshwater fishes of the Hudson Bay Drainage, Ungava Bay and the Arctic Archipelago. Pp. 53–104 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. Wiley, New York.

DeSalle, R., T. Freedman, E. M. Prager, and A. C. Wilson. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. Journal of Molecular Evolution 26:157–164.

Dunton, K. 1992. Arctic biogeography: the paradox of the marine benthic fauna and flora. Trends in Ecology and Evolution 7:183–189.

Durham, J. W., and F. S. MacNeil. 1967. Cenozoic migrations of marine invertebrates through the Bering Strait region. Pp. 326–349 *in* D. M. Hopkins, ed. The Bering land bridge. Stanford University Press, Stanford, Calif.

Esposti Degli, M., S. De Vries, M. Crimi, A. Ghelli, T. Patarnello, and A. Meyer. 1993. Function of mitochondrial cytochrome *b*, the best known membrane haemoprotein, expanded by DNA sequence analysis. Biochimica et Biophysica Acta 1143:243–271.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17:368–376.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

———. 1991. PHYLIP (Phylogeny Inference Package), version 3.42. University of Washington, Seattle.

Ferris, S. D., R. D. Sage, E. M. Prager, U. Ritte, and A. C. Wilson. 1983. Mitochondrial DNA evolution in mice. Genetics 105:681–721.

Finnerty, J. R., and B. A. Block. 1992. Direct sequencing of mitochondrial DNA detect highly divergent haplotypes in blue marlin (*Makaira nigricans*). Molecular Marine Biology and Biotechnology 1:206–214.

Foster, J. B. 1965. The evolution of the mammals of the Queen Charlotte Islands, British Columbia. British Columbia Provincial Museum Occasional Papers 14:1–130.

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

Grant, W. S. 1986. Biochemical genetic divergence between Atlantic, *Clupea harengus*, and Pacific, *C. pallasi*, herring. Copeia 1986:714–719.

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

Grant, W. S., and F. M. Utter. 1984. Biochemical population genetics of Pacific herring (*Clupea pallasi*). Canadian Journal of Fisheries and Aquatic Sciences 41:856–864.

Grantham, R. 1974. Amino acid difference formula to help explain protein evolution. Science 185:862–864.

Graves, J. E., and A. E. Dizon. 1989. Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (*Thunnus alalunga*). Canadian Journal of Fisheries and Aquatic Sciences 41:870–873.

Graves, J. E., S. D. Ferris, and A. E. Dizon. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonis pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Marine Biology 79:315–319.

Gross, H. P. 1978. Natural selection by predators on the defensive apparatus of the threespine stickleback, *Gasterosteus aculeatus* L. Canadian Journal of Zoology 56:398–413.

Gyllensten, U. B., and H. A. Erlich. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the *HLA-DQa* locus. Proceedings of the National Academy of Sciences, USA 85:7652–7655.

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

Haglund, T. R., D. G. Buth, and D. M. Blouw. 1990. Allozyme variation and the recognition of the "white stickleback." Biochemical Systematics and Ecology 18:559–563.

Haglund, T. R., D. G. Buth, and R. Lawson. 1992. Allozyme variation and phylogenetic relationships of Asian, North American, and European populations of the threespine stickleback, *Gasterosteus aculeatus*. Copeia 1992:432–443.

———. 1993. Allozyme variation and phylogenetic relationships of Asian, North American, and European populations of the ninespine stickleback, *Pungitius pungitius*. Pp. 438–452 *in* R. L. Mayden, ed. Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford, Calif.

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

Hillis, D. M., and C. Moritz. 1990. An overview of applications of molecular systematics. Pp. 502–515 *in* D. M. Hillis and C. Moritz, ed. Molecular systematics. Sinauer, Sunderland, Mass.

Hocutt, C. H., and E. O. Wiley, eds. 1986. The zoogeography of North American freshwater fishes. Wiley, New York.

Hubbs, C. L. 1929. The Atlantic American species of the fish genus *Gasterosteus*. Occasional Papers of the Museum of Zoology University of Michigan 200:1–9.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome *b* of mammals. Journal of Molecular Evolution 32:128–144.

Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256:100–102.

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

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals. Proceedings of the National Academy of Sciences, USA 86:6196–6200.

Lauder, G. V., and K. F. Liem. 1983. The evolution and interrelationships of the Acanthopterygian fishes. Bulletin of the Museum of Comparative Zoology 150:95–197.

Li, W.-H. 1993. Unbiased estimation of the rates of synonymous and nonsynonymous substitution. Journal of Molecular Evolution 36:96–99.

Lindsey, C. C., and J. D. McPhail. 1986. Zoogeography of fishes of the Yukon and Mackenzie Basins. Pp. 639–674 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. Wiley, New York.

Maggs, C. A. 1990. Distribution and evolution of noncoraline crustose red algae in the north Atlantic. Pp. 241–264 *in* D. J. Garbary and G. R. South, eds. Evolutionary biogeography of the marine algae of the North Atlantic. NATO ASI Series, Berlin.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Publications, Cold Spring Harbor, N.Y.

Martin, A. P., G. J. P. Naylor, and S. R. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357:153–155.

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): Morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62: 1402–1408.

———. 1994. Speciation and the evolution of reproductive isolation in sticklebacks (*Gasterosteus*) of south-western British Columbia. Pp. 399–437 *in* Bell and Foster 1994b.

McPhail, J. D., and C. C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Bulletin of the Fisheries Research Board Canada 173:1–381.

———. 1986. Zoogeography of the freshwater fishes of Cascadia (The Columbia System and Rivers North to the Stikine). Pp. 615–638 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. Wiley, New York.

McVeigh, H. P., S. E. Bartlett, and W. S. Davidson. 1991. Polymerase chain reaction/direct sequence analysis of the cytochrome *b* gene in *Salmo salar*. Aquaculture 95:225–233.

Meyer, A. 1993a. Evolution of mitochondrial DNA of fishes. Pp. 1–38 *in* P. W. Hochachka and T. P. Mommsen, eds. Biochemistry and molecular biology of fishes, vol 2. Elsevier, Amsterdam.

———. 1993b. Molecular phylogenetic studies of fishes. Pp. 219–249 *in* A. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman and Hall, London.

Meyer, A., and A. C. Wilson. 1990. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. Journal of Molecular Evolution 31:359–364.

Meyer, A., T. D. Kocher, P. Basasibwaki, and A. C. Wilson. 1990. Monophyletic origin of 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 of 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 threespine stickleback. Heredity 28:155–167.

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

Moritz, C., T. E. Dowling, and W. M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics 18:269–292.

Murphy, R. W., J. W. Sites, D. G. Buth, and C. H. Haufler. 1990. Proteins. I. Isozyme electrophoresis. Pp. 45–126 *in* D. M. Hillis and C. Moritz,

eds. Molecular systematics. Sinauer, Sunderland, Mass.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

Neigel, J. E., and J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515–534 *in* E. Nevo and S. Karlin, eds. Evolutionary processes and theory. Academic Press, London.

O'Reilly, P., T. E. Reimchen, R. Beech, and C. Strobeck. 1993. Mitochondrial DNA in *Gasterosteus aculeatus* and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution 47(2):678–684.

Ogilvie, R. T. 1989. Disjunct vascular flora of northwestern Vancouver Island in relation to Queen Charlotte Island's endemism and Pacific coast refugia. Pp. 127–130 *in* G. G. E. Scudder and N. Gessler, eds. The outer shores. Queen Charlotte Island Museum Press, Queen Charlotte Islands, British Columbia.

Pääbo, S. 1990. Amplifying ancient DNA. Pp. 159–169 *in* M. A. Innis et al., eds. PCR protocols: a guide to methods and applications. Academic Press, New York.

Palumbi, S. R., and B. D. Kessing. 1991. Population biology of the Trans-Arctic Exchange: mtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution 45:1790–1805.

Pamilo, P., and N. O. Bianchi. 1993. Evolution of the *Zfx* and *Zfy* genes: rates and independence between the genes. Molecular Biology and Evolution 10:271–281.

Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution 5:568–583.

Penczak, T. 1966. Comments on the taxonomy of the threespine stickleback, *Gasterosteus aculeatus* Linnaeus. Ohio Journal of Science 66:81–87.

Quinn, T. P., and J. T. Light. 1988. Occurrence of threespine sticklebacks (*Gasterosteus aculeatus*) in the open North Pacific Ocean: migration or drift? Canadian Journal of Zoology 67:2850–2852.

Rafinski, J., J. Banbura, and M. Przybylski. 1989. Genetic differentiation of freshwater and marine sticklebacks, (*Gasterosteus aculeatus*) of Eastern Europe. Zeitschrift fuer Zoologische Systematik und Evolutionsforschung 27:33–43.

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

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

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–525.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

Schofield, W. B. 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 Island Museum Press, Queen Charlotte Islands, British Columbia.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Smith, P. J., A. J. Birley, A. Jamieson, and C. A. Bishop. 1989. Mitochondrial DNA in the Atlantic cod, *Gadus morhua*: lack of genetic divergence between eastern and western populations. Journal of Fish Biology 34:369–373.

Sturmbauer, C., and A. Meyer. 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. Nature 358:578–581.

Swofford, D. L. 1991. Phylogenetic analysis using parsimony (PAUP), Version 3.0s. Illinois Natural History Survey, Champaign, Ill.

Taberlet, P., A. Meyer, and J. Bouvet. 1992. Unusual mitochondrial DNA polymorphism in two local populations of the blue tit, *Parus caeruleus*. Molecular Ecology 1:27–37.

Tajima, F. 1983. Evolutionary relationships of DNA sequences in finite populations. Genetics 122:437–460.

Thomas, W. K., and A. T. Beckenbach. 1989. Variation in salmonid mitochondrial DNA: evolutionary constraints and mechanisms of substitution. Journal of Molecular Evolution 29:233–245.

Vermeij, G. 1989. Geographical restriction as a guide to the causes of extinction: the case of the cold northern oceans during the Neogene. Paleobiology 15:335–356.

———. 1991. Anatomy of an invasion: the trans-Arctic interchange. Paleobiology 17:281–307.

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.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26:375–400.

Wilson, A. C., H. Ochman, and E. M. Prager. 1987. Molecular time scale for evolution. Trends in Genetics 3:241–247.

Withler, R. E., and J. D. McPhail. 1985. Genetic variability in freshwater and anadromous sticklebacks (*Gasterosteus aculeatus*) of southern British Columbia. Canadian Journal of Zoology 63:528–533.

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

———. 1984. A functional biology of the sticklebacks. University of California Press, Berkeley.

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