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Mitochondrial DNA evidence of an early Holocene population expansion of threespine sticklebacks from Scotland

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Abstract

In this study, we analyzed the cytochrome *b* gene in threespine stickleback (*Gasterosteus aculeatus*) populations from Scotland. We found evidence of a postglacial population expansion in Scotland and large differences in genetic diversity estimates among populations. Higher levels of genetic diversity are negatively correlated with distance from the ocean. In addition, distance from the ocean and predation risk both explain variation in plate count in Scottish populations. Overall, the mtDNA data support the racemic model of evolution in threespine stickleback.

Keywords: Genetic structure; Haplogroup; Migration; *Gasterosteus aculeatus*; Cytochrome *b*; Gene flow; Population expansion; Stickleback

1. Introduction

Following the retreat of the glaciers during the early Holocene (~12,000 yBP), some marine fishes independently colonized freshwater environments throughout the Northern Hemisphere. Threespine stickleback (*Gasterosteus aculeatus*) populations rapidly adapted to diverse freshwater environments resulting in unprecedented phenotypic diversity. Presently, some freshwater populations continue to experience gene flow from marine populations through interbreeding with anadromous sticklebacks (Taylor and McPhail, 1999).

Their evolutionary history and propensity for rapid, adaptive evolution (Bell and Foster, 1994; Bell et al., 2004) make the threespine stickleback an excellent system for studying parallel evolution. Such studies have become increasingly sophisticated with molecular tools that are allowing us to determine whether similar phenotypes evolved from the same changes in genetic mechanisms (Colosimo et al., 2005; Nachman, 2005). However, the study of parallel evolution requires independently evolving populations. To determine the degree of independence among different populations, we need to understand the history of stickleback populations. Specifically, the question of whether similar allelic patterns in noncontiguous regions are the result of common ancestry, migration or selection cannot be convincingly resolved without this information.

Several studies have examined the genetic diversity of threespine sticklebacks (Deagle et al., 1996; Haglund et al., 1992; Higuchi and Goto, 1996; Higuchi et al., 1996; Johnson and Taylor, 2004; Ortí et al., 1994; Raeymaekers et al., 2005; Reusch et al., 2001; Taylor and McPhail, 1999; Watanabe et al., 2003). Ortí et al. (1994) provided the first comprehensive study by investigating diversity within the mitochondrial cytochrome *b* gene in a global survey. Ortí et al. found two ancient clades, one in Japan and along the west coast of North America, the Trans North Pacific Clade, TNPC, originally named by (Thompson et al., 1997),

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and the other in populations from the west and east coast of North America and on the other side of the Atlantic basin in Europe (the Euro-American Clade (EAC)). Combined with fossil evidence, they concluded that the Atlantic clade was derived from the Pacific clade, and that this, in part, contributed to the reduced genetic variation among Atlantic populations. However, there is scope for additional mitochondrial DNA (mtDNA) information about Atlantic sticklebacks because unlike their freshwater Pacific counterparts (Taylor and McPhail, 1999), Atlantic populations were under-represented in population genetic surveys.

On a regional level, Raeymaekers et al. (2005) recently analyzed multilocus genotypes, STRs and allozymes to investigate the genetic structure of stickleback populations in Northern Europe. They identified data consistent with the 'racemic model' of evolution which posits that many freshwater populations independently emerged from marine/anadromous ancestors during the early Holocene, a period of postglacial retreat that resulted in a population expansion and colonization of freshwater habitats (Bell, 1976, 1986). However, the results from Raeymaekers et al. (2005) are also consistent with a model of differential gene flow (low gene flow among freshwater populations and high gene flow among anadromous populations) and a lower effective population size among freshwater populations.

The analysis of additional genetic systems can help determine if the genetic structure of stickleback populations in the North Atlantic were shaped by a population expansion. The use of mtDNA is a powerful tool for detecting past demographic events because the effective population is one fourth of that in the nuclear genome and therefore mtDNA is more sensitive to past demographic events. In addition, because mitochondrial genome is nonrecombining, we can trace the ancestry and relationships among haplotypes. A particularly useful tool for visualizing relationships among haplotypes are networks, which provide a meaningful representation of the genealogical relationships among individuals in recently diverged groups that is unattainable in the analysis of phylogenetic trees. Therefore, to investigate the genetic structure and population history of stickleback populations in Scotland, we sequenced the cytochrome *b* gene of 224 threespine sticklebacks from 1 marine and 11 freshwater populations. For comparative purposes, we also sequenced 11 individual ninespine sticklebacks (*Pungitius pungitius*).

Following the Loch Lomond Stadial at 11,000–10,000 rcybp, glaciers began to retreat in Scotland as temperatures rose dramatically. By around 7500 ybp the temperature in Scotland was about what it is today (Sissons, 1979). During the approx. 2500 years of glacial melt, a reasonable hypothesis is that more northern populations were covered in ice for longer (and are hence younger) than more southern populations.

Our results suggest two general conclusions. First, there was a postglacial population expansion in Scotland. Second, regional genetic structure in Scottish populations is primarily influenced by access to the ocean. These two lines of evidence add support to the 'racemic model' of stickleback evolution. Although here, we are primarily concerned with the pattern of genetic variation among populations, we also report data on lateral plate counts in the different populations. We report preliminary analyses on the relative influence of gene flow and local predation pressure, which has been identified as an important selective factor on variation in plate number (Bell and Richkind, 1981; Hagen and Gilbertson, 1972, 1973; Moodie et al., 1973; Reimchen, 1995).

2. Materials and methods

As part of a larger study, sticklebacks were collected from 11 different freshwater lochs in Scotland (Fig. 1). The intensity of predation by piscivorous fish (pike, perch, brown trout, and eels) was assessed using historical records and whole-lake seine surveys. Populations were classified as 'low risk' if they did not contain any piscivorous fishes, and were classified as 'high risk' if piscivorous fishes were detected (Table 2). Although sticklebacks are also subject to predation by birds and invertebrate larvae, those predators can move between water-bodies, making it difficult to determine predation risk by those predators. We classified populations greater than 14 km from the ocean as 'inland' and populations within 14 km of the ocean as 'coastal'. For comparison, one marine population was sampled near the island of Great Cumbrae, and one ninespine stickleback population was sampled on the Isle of Bute. Sticklebacks were collected between 11 August and 25 September 2004. Fish were transported to the laboratory in insulated buckets and maintained at Glasgow University Field Station. Fish were killed by a method approved by the home office. Samples for DNA were taken by fin clip from the caudal fin of dead fish. The number of lateral plates on alcohol-preserved specimens along the left side of the body was counted using a dissecting prod under a dissecting microscope.

DNA was extracted from 224 tissue samples from threespine sticklebacks and 11 samples from ninespine sticklebacks. Approximately 25 mg of each sample was used for the extraction with the Qiagen DNAEasy Tissue Kit®. A 1210 base pair (bp) region of the cytochrome *b* gene was amplified using primers Stkl14301 (CCTACCAGGACTTT AACCAGGACTA) and Stkl15511 (CCGGCGCTCTGG CGCTGAGCACTTT). The DNA was sequenced in both forward and reverse directions and a contig was created by combining forward and reverse sequences based on a large region of overlap. PCR amplification was performed in 20 μl volumes with 0.5 μM primers, 0.2 μM dNTPs, 2.5 mM $MgCl_2$, 1× Invitrogen PCR buffer (2 μl of 20× buffer, which consists of 200 mM Tris–HCl (pH 8.4) and 500 mM KCl), 0.3 U platinum Taq polymerase and 25–100 ng of template DNA. Amplification was carried out on Eppendorf Mastercycler thermal cyclers following an initial three minute step