Resolution of population structure in a species with high gene flow: microsatellite variation in the eulachon (Osmeridae: Thaleichthys pacificus)

Abstract Five microsatellite loci were used to examine genetic variation within and among putative populations of the eulachon, Thaleichthys pacificus (Pisces: Osmeridae), over the entire range of the species. A previous mitochondrial DNA study, while revealing a high degree of genetic variation within the species, did not resolve the level of population sub-division expected for this anadromous fish. Two microsatellite loci were developed from eulachon DNA and, in addition to three microsatellite loci from the rainbow smelt, Osmerus mordax, were employed as a class of “higher resolution” markers in an attempt to further resolve the population structure of eulachon. The level of genetic variation observed at these loci was surprisingly low (heterozygosity ranged from 4% to 64%; number of alleles ranged from three to ten; maximum size range of alleles was 16 base pairs), yet revealed the greater power of microsatellites over mitochondrial DNA for resolving population sub-division within eulachon. More pairwise population comparisons were significant with the microsatellite data, and the microsatellite $F_{ST}$ value was twice the value observed with mtDNA (mtDNA $F_{ST}=0.023$; microsatellite $F_{ST}=0.045$). Despite this greater sensitivity, it was difficult to define distinct demographic units in eulachon, a species which is currently the focus of conservation concern. Eulachon highlight the challenges of examining population structure in species with inferred high gene flow.

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J.E. McLean (✉)
School of Aquatic and Fishery Sciences,
University of Washington, Seattle, WA 98195, USA
E-mail: jem34@u.washington.edu
Fax: +1-206-6857471

E.B. Taylor
Department of Zoology and Native Fish Research Group, University of British Columbia,
Vancouver, BC, V6T 1Z4, Canada

Introduction

The potential for migration and gene flow among populations varies widely over taxa. The potential for dispersal of fishes can be divided into three basic life history categories (freshwater, anadromous and marine) that encompass both the capacity for gene flow among populations and its reflection in neutral genetic variation. The proportion of the total genetic variance that can be attributed to differences among populations, $F_{ST}$, differs among these categories (Ward et al. 1994). Values of $F_{ST}$ based on allozymes averaged 0.222 among freshwater fish populations commonly limited to specific lakes or drainages, and having restricted contact with other populations of the same species. Values of $F_{ST}$ in anadromous species with higher dispersal and mixing potential averaged 0.108. Among marine species with the highest potential for migration and gene flow among populations, average $F_{ST}$ values were 0.062. Although their environment plays a significant role by facilitating access to other populations, their often large population sizes are also involved in maintaining high within-population variation and low among-population divergence in marine fish (Gyllensten 1985). Marine species such as cod, hake, herring and squid have previously shown little population sub-division, and have been thought to be homogeneous over large geographic ranges; however, closer investigation with microsatellite loci has recently revealed population structure (Bentzen et al. 1996; O’Connell et al. 1998; Lundy et al. 1999; Shaw et al. 1999).

Microsatellite loci form a class of highly polymorphic, and therefore highly informative, nuclear DNA, and have proved an effective class of markers for studies of intraspecific population structure, as well as hybridization, linkage mapping, paternity testing and pedigree analysis (Amos et al. 1993; Queller et al. 1993; Bowcock et al. 1994; Roy et al. 1994; Dowling et al. 1997). Microsatellites are known as a class of “high resolution” markers due, in part, to their ability to resolve intra-

Eulachon (Osmeridae: \textit{Thaleichthys pacificus}) have a distribution limited to the west coast of North America, and range from the southern Bering Sea to the Columbia River (Hay 1996) (Fig. 1). Throughout this range, they spawn in only 20 to 30 rivers, and appear to prefer rivers with a glacial influence and a spring freshet (Hay 1996). Although they are anadromous, they spend very little time in freshwater, either at the beginning or the end of their lives. After spending 3 or 4 years in the ocean, mature adults migrate to freshwater and spawn a very short distance upriver, often remaining within the tidal influence, and they are thought to die after spawning.

Fertilized eggs attach to the substrate and hatch after 2–3 weeks. After hatching, larvae are washed out of the river within 24 h. Because eulachon spend so little time in freshwater, it is possible that they are not as dependent on specific freshwater habitats as other anadromous species, for example, Pacific salmon which are often highly structured genetically (Taylor et al. 1994; Allendorf and Waples 1996; Wenburg et al. 1998). This putative characteristic is consistent with the results of our previous mitochondrial DNA (mtDNA) study, which revealed a number of weakly sub-divided populations throughout the range of eulachon not structured on a river-by-river basis (McLean et al. 1999). Further, eulachon exhibit a “wandering” characteristic. They will spawn in one location consistently for a number of years, and then will disappear for a year, appearing in a river in which they have not previously been observed (Hay 1996). Because eulachon are anadromous, it was expected that they would consist of populations that are structured river-by-river, and would exhibit an intermediate $F_{ST}$ value such as that observed in other anadromous species. Although the mtDNA data failed to reveal the level of population structure generally expected of anadromous fish, a higher resolution marker such as microsatellites may reveal population sub-division on a finer scale.

The primary objective of this study was to describe microsatellite variation in eulachon, and to complete an analysis of population structure and compare the results from this project to those from our previous mtDNA study. Further, as eulachon numbers have declined dramatically in recent years and eulachon have become a conservation concern (Hay 1996), we wanted to examine potential conservation units. As an anadromous species, eulachon population units may be isolated physically and/or behaviorally; and here we examine whether such isolation has generated population structure in eulachon and if this structure fits an island model or an isolation-by-distance model.

**Materials and methods**

**Sample collection**

Samples used for this microsatellite analysis were a subset of the samples used in the previous mtDNA analysis (McLean et al. 1999), and were collected from twelve locations that span the entire geographic distribution of the species (Fig. 1). These locations include eleven freshwater sites and one marine site (Bering Sea). Replicate year samples were collected at two freshwater locations (Table 1). The total sample size for the microsatellite analysis was 293 individuals. Samples were taken as either livers or dorsal fins and stored in 95% ethanol until DNA was extracted.

**DNA extraction**

Genomic DNA was obtained from tissue samples by Pronase digestion/phenol-chloroform extraction (Taggart et al. 1992). The DNA was resuspended in 20–100 μl of TE solution (10 mM Tris, 1 mM EDTA in H$_2$O; pH 8.0) and stored at –20°C.