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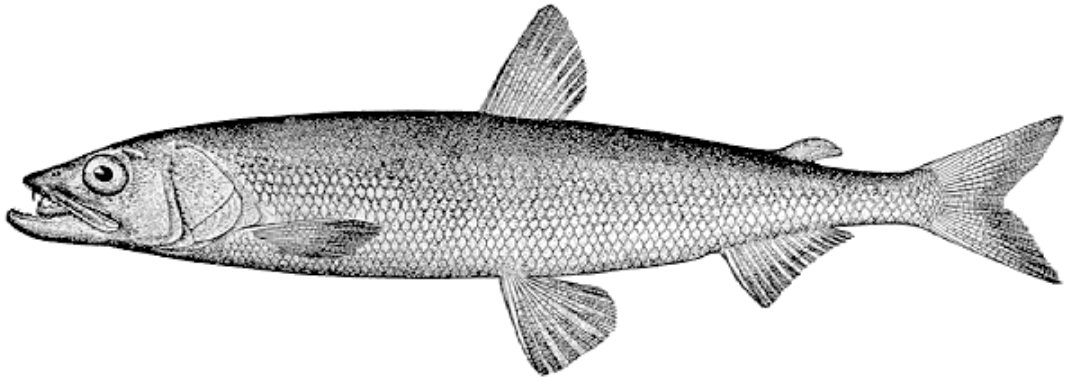

Conservation Genetics Laboratory
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Blair G. Flannery, Cheryl A. Anderson, Cara J. Lewis, and John K. Wenburg

Abstract

Rainbow smelt *Omerus mordax* return to the Togiak River at varying times throughout the year. Genetic variation at nine microsatellite loci was analyzed to determine whether winter and spring runs were reproductively isolated. Results revealed that the winter and spring collections had moderate to high levels of genetic diversity. However, no genetic divergence (P>0.05) was observed between the collections. The collections likely come from a single, randomly mating (panmictic) population.

Introduction

Rainbow smelt *Omerus mordax* are anadromous and return to the Togiak River in the fall, winter, late spring and early summer. The late spring and early summer returns are expected as they coincide with known spawning times. It is unknown if the fall and winter returns have distinct spawning, but it is plausible as spawning is known to range from March to July among populations of rainbow smelt in eastern Canada (Scott and Scott 1988). Such variation in reproductive timing suggests that extended or bimodal spawning may be probable in rainbow smelt populations (Coulson et al. 2006).

Reproductive timing is a heritable trait in salmonids (Hendry and Day 2005 and references therein). This heritability provides a mechanism for reducing gene flow temporally in species with extended reproductive times in a manner analogous to geographic distance (isolation by distance; Wright 1943). The concept of temporal genetic divergence has recently been termed isolation by time (Hendry and Day 2005). Less is known about the heritability of reproductive timing in non-salmonids, but there is evidence that it is under some genetic control in rainbow smelt (Rupp and Redmond 1966). In this pilot study, we analyzed genetic variation at nine microsatellite loci in winter and spring collections of Togiak River rainbow smelt to quantify the genetic divergence between them.

Methods

Genetic samples were collected from rainbow smelt returning to the Togiak River in the spring of 2007 and the winter of 2008 (Table 1; Dion and Bromaghin 2008). Samples were genotyped at nine microsatellite loci: *Tpa104*, *Tpa111*, *Tpa112*, *Tpa113*, *Tpa114*, *Tpa115*, *Tpa118*, *Tpa127*, and *Tpa129* (Kaukinen et al. 2004). The data were checked for

Authors: Blair G. Flannery, Cara J. Lewis, and John K. Wenburg may be contacted at the U.S. Fish and Wildlife Service, Conservation Genetics Laboratory, 1011 E. Tudor Rd., Anchorage, AK 99503. Cheryl A. Anderson may be contacted at the U.S. Fish and Wildlife Service, Anchorage Fish and Wildlife Field Office, 605 W. Fourth Ave., Room G-61, Anchorage, AK 99501.

duplicated genotypes using the program MICROSATELLITE TOOLKIT (Park 2001), and any duplicates were removed. Where multiple tests of the same hypothesis were performed, a sequential Bonferroni method was used to maintain the overall alpha at 0.05 (Rice 1989).

The genetic data were analyzed for conformance to Hardy-Weinberg and gametic phase equilibrium using the computer programs FSTAT 2.9.3 (Goudet 2001) and GENETIX 4.05 (Belkhir et al. 1996), respectively, to determine if the samples represented randomly mating populations. The computer program MICRO-CHECKER (Oosterhout et al. 2004) was used to assess significant deviations from Hardy-Weinberg equilibrium. In addition, FSTAT 2.9.3 and GENETIX 4.05 were used to calculate estimates of allelic richness, percentage polymorphic loci (95%), and observed and expected heterozygosity.

An unbiased gene diversity analysis was used to estimate the degree of divergence between the seasonal returns based on the relative measure, G_{ST} (Chakraborty and Leimar 1987; Nei and Chesser 1983). Significance of the G_{ST}-statistics was inferred from likelihood ratio (*G*-test, Sokal and Rohlf 1995) tests of allele frequency homogeneity (Chakraborty and Leimar 1987). Alleles were pooled if overall expected counts were less than three in order to maintain the *G*-test's approximation of the χ^2 probability distribution (Sokal and Rohlf 1995). In addition, the computer program GENEPOP 4.0 (Raymond and Rousset 1995) was used to conduct Fisher's exact tests of genotypic frequency homogeneity.

Results and Discussion

Two loci (*Tpa112* and *Tpa127*) were out ($P<0.05$) of Hardy-Weinberg equilibrium in both the winter and spring collections. These loci had an excess of homozygotes that were likely caused by null alleles. Using a maximum likelihood algorithm in ML-NULLFREQ (Kalinowski and Taper 2006), the allele frequencies for the null alleles were estimated and used in the gene diversity and likelihood ratio analyses. All loci were in gametic phase equilibrium.

Loci ranged in allele number from 4-36, in allelic richness from 2.5-30.0, and in expected heterozygosity from 0.260-0.908 (Table 2). All loci were polymorphic at the 95% criterion in the collections, and the collections had similar levels of allelic richness, 11.1-11.4, and expected heterozygosity, 0.580-0.594 (Table 3). These estimates of variation indicate that the collections contain moderate to high levels of genetic diversity (Frankham et al. 2002).

The gene diversity analysis indicated that individuals varying within collections accounted for 99.93% of the variation, while between collections accounted for 0.07% (Table 4). However, the G_{ST} value (0.07%) was not significant ($P>0.05$). Significant genetic divergence was not observed between the collections (Table 5). The likelihood ratio analysis of allele frequencies and the Fisher's exact test of genotypic frequencies gave similar results. One locus, *Tpa113*, had a *P*-value below 0.05, but this was not judged significant after sequential Bonferroni correction.

The absence of detectable genetic divergence between the winter and spring collections suggests that they are from the same spawning population. If spawning does occur continuously from March through June, which spans the sampling periods for the winter and spring collections, then the absence of divergence likely results from gene flow through temporal straying, with the assumption that reproductive timing is heritable (Coulson et al. 2006), though local adaptation to spawning time can still occur with little influence on neutral allele frequencies (Hawkins et al. 2002). Genetic divergence can occur within a river between early and late spawning times if there is sufficient temporal isolation and short spawning duration (≤3 nights) by individual fish (Coulson et al. 2006).

Coulson et al. (2006) observed divergence in rainbow smelt in a stream when spawning times were separated by a month, but not when spawning occurred continuously from the early to late time periods. Moreover, Coulson et al. (2006) suggest that spawning duration by individual fish may be the most important factor in the formation of temporal genetic divergence. Their simulations indicate that divergence is possible with as little as a two week separation between spawning times if individuals spawn on only one night, but not if individuals spawn for more than three nights, even with spawning times separated by over a month.

Summary

- The rainbow smelt in the Togiak River have moderate to high within population genetic diversity.
- The early and late rainbow smelt collections are not significantly divergent.
- These collections of rainbow smelt from the Togiak River likely come from a single, randomly mating (panmictic) population.

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Table 1. Sample: location, seasonal run, year collected, sampling period, and number (N) of samples collected.

Location	Seasonal Run	Year	Sampling Period	N
Togiak River	Spring	2007	5/16 - 6/18	200
Togiak River	Winter	2008	3/1 - 4/29	175

Table 2. Results across collections for each locus: number of alleles, allelic richness (A_R), unbiased expected heterozygosity (H_E), and observed heterozygosity (H_O).

Locus	No. Alleles	A_R	H_E	H_O
Tpa112	7	5.6	0.331	0.268
Tpa113	13	11.9	0.773	0.729
Tpa114	36	30.0	0.908	0.926
Tpa115	4	2.5	0.501	0.493
Tpa129	15	14.0	0.868	0.889
Tpa104	7	5.6	0.563	0.567
Tpa111	6	5.6	0.346	0.342
Tpa118	7	5.1	0.260	0.257
Tpa127	31	21.2	0.730	0.645

Table 3. Results for each collection across all loci: mean number of samples successfully genotyped (N), percentage polymorphic loci at the 95% criterion ($\%P$), allelic richness (A_R), unbiased expected heterozygosity (H_E), and observed heterozygosity (H_O).

Location	Seasonal Run	N	$\%P$	A_R	H_E	H_O
Togiak River	Spring	191	100	11.4	0.594	0.585
Togiak River	Winter	163	100	11.1	0.580	0.552

Table 4. Unbiased gene diversity analysis based on nine microsatellite loci.

Source of variation	Gene diversity	G_{ST}-statistics
Average within season	H_S=0.6051	H_S/H_T=0.9993
Total gene diversity	H_T=0.6055	G_{ST}=0.0007

Table 5. Tests of homogeneity based on nine microsatellite loci.

	Likelihood Ratio			Exact Test
	df	G	P	P
Tpa112	3	2.08	0.56	0.57
Tpa113	7	14.50	0.04	0.02
Tpa114	18	17.07	0.52	0.50
Tpa115	1	0.11	0.73	0.94
Tpa129	11	11.53	0.40	0.39
Tpa104	3	0.34	0.95	0.95
Tpa111	3	1.06	0.79	0.24
Tpa118	2	0.44	0.80	0.68
Tpa127	12	17.41	0.13	0.41
Overall	60	64.54	0.32	0.43