Assessment of Gene Flow in Pacific lamprey using Microsatellite Markers

Recent genetic data suggests that Pacific lamprey could be weakly philopatric. Goodman et al (2008) found little evidence of genetic differentiation among Pacific lamprey along the Pacific Coast of North America using mitochondrial DNA markers, which could be explained by lack of homing. In contrast, we found that adult Pacific lamprey across their range were genetically differentiated and that that gene flow among aggregations of Pacific lampreys from their natal region to other regions decreased as the distance to those locations increased (Lin et al. 2008a, Lin et al. 2008b). This indicated some greater degree of philopatry. We noted that this level of philopatry could occur through attraction mechanisms such as migratory pheromones or stream flows as long as ocean migration distances were restricted and habitats and aggregations where lamprey historically occurred remained intact (Lin et al. 2008a; Lin et al. 2008b).

Recent concerns were raised by fisheries management agencies about the reintroduction of adults and/or juvenile lamprey in the upper Columbia River basin to assist in Pacific lamprey restoration. We propose to use microsatellite markers to clarify or define populations or aggregations in the Columbia River and along the West coast of North America. This proposal is for a two year study and is a key element in the CRITFC tribes' Pacific lamprey restoration plan for the Columbia River basin. We will share information with Dr. Margaret Docker who is also pursuing genetic studies specific to the Willamette River on behalf of CRITFC.

Objective 1: Isolate polymorphic microsatellite markers using Fast Isolation by AFLP of Sequences Containing repeats (FIASCO) and bioinformatics analyses.

Task 1. Isolate, clone, sequencing and bioinformatics analyses of 130 candidate microsatellite markers of Pacific lamprey.

Task 2. Design primers based on the results of task 1. We will use primers for PCR to amplify microsatellite sequences to discriminate polymorphic and monomorphic candidate microsatellite markers from Pacific lamprey samples already collected.

Approach:

Amplified fragment length polymorphism (AFLP) is a technique developed for genomic DNA fingerprinting (Vos et al., 1995). It combines the techniques of restriction endonuclease digestion and polymerase chain reaction (PCR) amplification of restriction fragments, and thus possesses the advantages of these two techniques. AFLP analysis has a higher resolution and sensitivity than RAPD analysis in revealing allelic polymorphism (Barker et al., 1999) and hence is suggested to be a good technique for studying genetic variation (Mueller and Wolfenbarger, 1999). This technique has been widely applied to study genotyping, population differentiation and genetic diversity in a wide variety of organisms, such as catfish (Liu et al., 1998), ayu (Seki et al., 1999), large yellow croaker (Wang et al., 2000) and most recently Pacific lamprey (Lin et al. 2008a; Lin et al. 2008b).

A novel AFLP based technique named as FIASCO (Fast Isolation by AFLP of Sequences Containing repeats) has been set up and demonstrated as a fast and simple method for microsatellite makers isolation in different organisms such as birds (*Passera lagia*), fish (*Sparus aurata* and *Lophius americanus*), crustacean (*Meganyctiphanes norvegica*) and red coral (*Corallium rubrum*) (Zane et al., 2002). This new technique will help us increase the number of polymorphic microsatellite loci for analysis of genetic variation within and geographic areas.

We have successfully used AFLP technique to assess the genetic diversity in Pacific lampreys (Lin et al. 2008a; Lin et al. 2008b). Our results are among the first to suggest genetic structure at DNA level in Pacific lamprey. Based on this work, we have conducted a small scale screening using FIASCO to isolate microsatellite markers in Pacific lamprey. From the small screening, two microsatellite markers were polymorphic out of 11. We have another 130 candidates in hand that need to be further characterized.

Objective 2: To estimate levels of genetic diversity and degree of spatial genetic differentiation among populations or aggregations of Pacific lamprey from the Columbia River Basin and rivers along the west coast of North America.

Task 2.1. Collect additional tissue samples and extract DNA from adult lampreys in Columbia River Basin and along the west coast. Approximately 20 samples from each river will be acquired for analyses.

Task 2.2. Estimate levels of genetic diversity among Pacific lamprey. Examine variations between years and multiple years at some geographic locations and temporal aspects of variations.

Approach:

We already have tissue samples from a previous AFLP analysis as described (Lin et al. 2008a; Lin et al. 2008b). Tissue samples were taken from adult Pacific lamprey from 4 locations of the Columbia River basin; Willamette River, OR, N. Fork Toutle River, WA, Deschutes River, OR, and John Day Dam, OR as well as two rivers along the coasts of Oregon; Rogue River, Klamath River, and one in Alaska (Fig. 1). We propose to collect more samples from adult Pacific lampreys in the Columbia River Drainage and rivers along the Pacific coast. Sites will include the Willamette, Deschutes, Klickitat, John Day, Yakima, Wenatchee, and North Fork Toutle rivers and 15 Mile Creek. Coastal rivers targeted will include the Alsea, Umpqua, Rogue, and Klamath. The additional sampling will be used to determine at what scale differences exist. Sampling will be coordinated with CRITFC and CRITFC's member tribes.

DNA will be extracted from Pacific lamprey tissue samples using the Qiagen DNeasy kit. DNA quantities will be assessed using flourimetry.

Each individual will be run on a gel with molecular weight standards of known size as well as individuals of known genotype previously run to maintain consistency in scoring across all gels. Additionally, a subset of individuals will be rerun and scored to ensure the same samples were scored consistently in independent runs. All gels will be scored independently by 2 individuals.

We will estimate allele frequencies for each locus and population and conduct exact tests for deviation from Hardy-Weinberg Equilibrium and for linkage disequilibrium, using program GENEPOP (Raymond & Rousset 1995). We will make estimates of statistically significant differences in allele frequencies among populations using F-statistics by using the program Arlequin (Schneider et al., 2000). Estimates of Cavalli-Sfoza-Edwards chord distance (Cavalli-Sforza and Edwards, 1967) will be obtained using the program PHYLIP (Felsenstein 1992). A Neighbor-joining phenogram summarizing pair-wise relationships among populations will be produced using the program PHYLIP. Estimates of statistical support for branching nodes will be based on 1000 bootstrap replicates.

Deliverables

One completion report, and possibly 2 peer reviewed articles.

References

Barker, J.H.A., Matthes, M., Arnold, G.M., Edwards, K.J., Ahman, I., Larsson, S., Karp, A., 1999.Characterization of genetic diversity in potential biomass willows (Salix spp.) by RAPD and AFLP analyses. Genome 42, 173–183.

Bryan MB, Libants SV, Warrillow JA, Li W and Scribner KT. 2002. Polymorphic microsatellite markers for the landlocked sea lamprey, *Petromyzon marinus.* In press from *Conservation Genetics.*

Cavalli-Sforza, L.L. and Edwards A.W.F. (1967) Phylogenetic analysis models and estimation procedures American Journal of Human Genetics 19: 233-257

Felsenstein J (1992) PHYLIP (*Phylogeny Inference Package*) Version 3.5C. Department of Genetics, SK-50, University of Washington, Seattle, 98195, USA.

Goodman, D. H., S. B. Reid, M. F. Docker, G. R. Haas, and A. P. Kinziger. 2008. Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). Journal of Fish Biology 72:400-417.

Liu, Z., Nichols, A., Li, P., Dunham, R.A., 1998. Inheritance and usefulness of AFLP markers in channel catfish (Ictalurus punctatus), blue catfish (I. furcatus), and their F1, F2 and backcross hybrids. Mol. Gen. Genet. 258, 260–268.

Lin, B.B., Zhang, Z.P., Wang, Y.L., Currens, K., Spidle, A., Yamazaki, Y., and Close, D.A., 2008a. AFLP Assessment of Genetic Diversity of Pacific Lamprey. *North American Journal of Fisheries Management*. 28:1182-1193. 6

Lin, B., Z. Zhang, Y. Wang, K.P. Currens, A. Spidle, Y. Yamazaki, and D.A. Close. 2008b. Erratum: AFLP assessment of genetic diversity of Pacific lamprey. North American Journal of Fisheries Management 28:1648.

Mueller, U.G., Wolfenbarger, L.L., 1999. AFLP genotyping and fingerprinting. Trends Ecol. Evol. 14, 389–394.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *J. Heredity*, 86, 248-249.

Seki, S., Agresti, J.J., Gall, G.A.E., Taniguchi, N., Bernie, M., 1999. AFLP analysis of genetic diversity in three populations of ayu Plecoglossus altivelis. Fish. Sci. 65, 888–892.

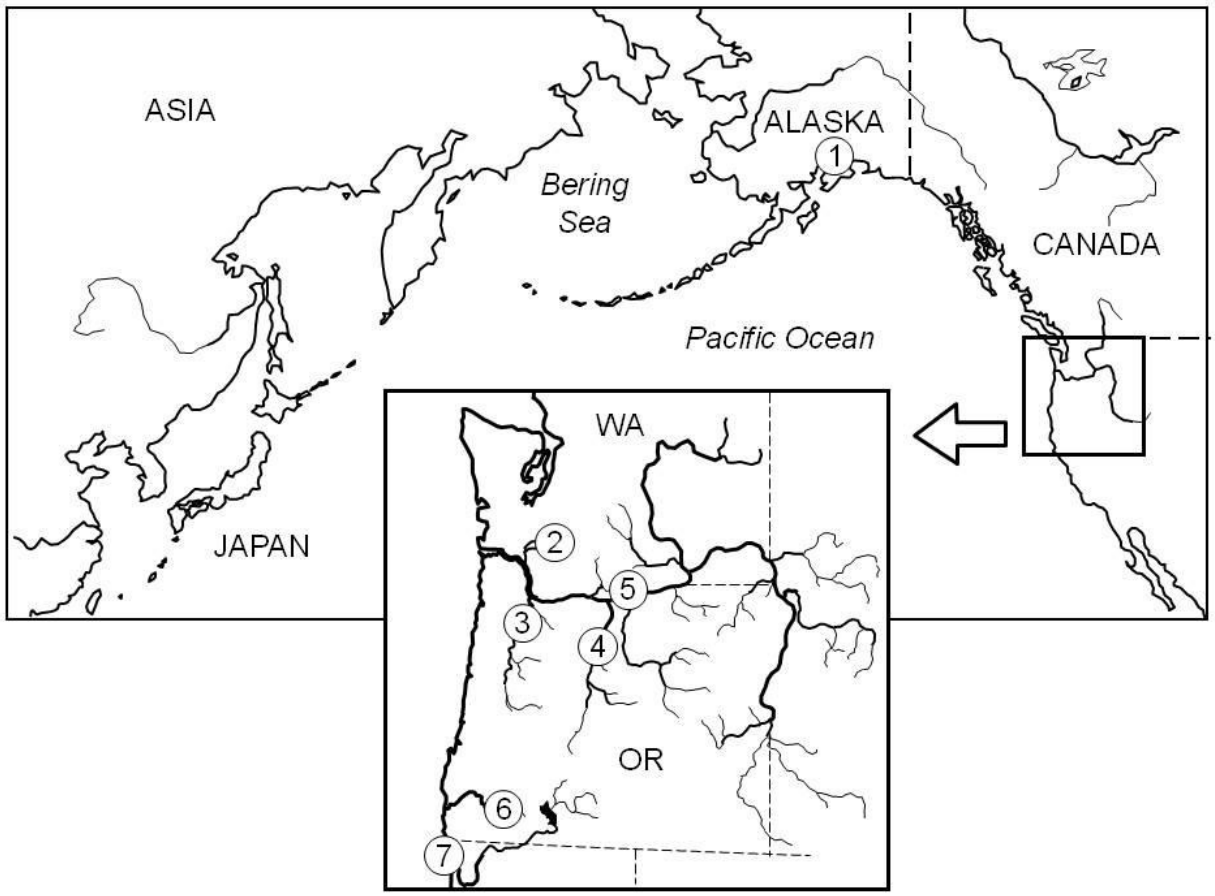
Schneider, S., Roessli, D., Excoffier, L. (2000) Arlequin ver 2.000 A software for population genetics data. Genetics and biometry laboratory, University of Geneva, Switzerland

Vos, P., Hogers, R., Bleeker, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23, 4407–4414.

Wang, Z.Y., Wang, Y.L., Lin, L.M., Khoo, S.K., Okamoto, N., 2002. Genetic polymorphisms in wild and cultured large yellow croaker Pseudosciaena crocea using AFLP fingerprinting. J. Fish. Sci. China. 9, 198-202.

Zane, L., Bargelloni, L., Patarnello, T. 2002. Strategies for microsatellite isolation: a review. Mol Ecol, 2002; 11(1): 1-16. 7

Figure 1. Locations of tissue samples collected from adult Pacific lampreys. 8



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