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Article *in* North American Journal of Fisheries Management · August 2008

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North American Journal of Fisheries Management 28:1182–1193, 2008

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Amplified Fragment Length Polymorphism Assessment of Genetic Diversity in Pacific Lampreys

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Abstract.—This study is the first to document genetic differences among Pacific lampreys *Lampetra tridentata* across much of their range. We examined collections of migrating adult Pacific lampreys from the Naka River, Japan; Moose River, Alaska; and six Pacific Northwest locations (North Fork Toutle, Willamette, Deschutes, John Day, Rogue, and Klamath rivers) based on variation at 180 polymorphic loci among the 556 amplified fragment length polymorphism loci generated by seven primer combinations. Despite the large geographical distances separating the samples, the different collections were characterized by a high proportion of shared bands, which indicated significant levels of historical gene flow across the range of the species. Analysis of molecular variance across three geographical regions—the Pacific Northwest, Alaska, and Japan—showed divergence among samples (genetic differentiation index $F_{ST} = 0.106$, $P < 0.001$) and significant differences among regions (regional differentiation $F_{RT} = 0.014$; $P < 0.001$), among Pacific Northwest collections (population differentiation $F_{SR} = 0.092$; $P < 0.001$), and within collections. Over this extent of the species' range, genetic divergence tended to follow a pattern of isolation by distance, which suggested that allelic diversity may have been maintained by stepping stone patterns of dispersal. This pattern did not occur within the Pacific Northwest: among the six collections, all pairwise F_{ST} comparisons were statistically significant and ranged from 0.037 to 0.182, but the differences corresponded to no obvious geographical pattern.

Pacific lampreys *Lampetra tridentata* are anadromous fish found mostly in coastal streams and major rivers of the North Pacific from the Aleutian Islands to Baja California and in Asia as far south as Hokkaido, Japan (Scott and Crossman 1973; Ruiz-Campos and Gonzalez-Guzman 1996). They migrate to sea at 3–7 years of age after metamorphosing from nonparasitic, filter-feeding ammocoetes to the parasitic migratory form. At sea for 6–40 months, they attach to other fishes and whales to feed and migrate (Pletcher 1963; Kan 1975; Beamish 1980). Although adults return to rivers to spawn, little is known about mechanisms that guide Pacific lampreys to return to freshwater, patterns of dispersal, or incidence of natal philopatry. Petromyzonol sulfate, a bile acid produced by lamprey ammocoetes that can be detected by migrating adults, has been documented in Pacific lampreys (Yun et al.

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Received February 23, 2007; accepted November 21, 2007
Published online August 21, 2008

2003; Fine et al. 2004), indicating that olfactory cues constitute one mechanism by which migrating adults choose spawning locations. A similar mechanism is used by sea lampreys *Petromyzon marinus*, which occur in Atlantic Ocean watersheds (Bjerselius et al. 2000; Vrieze and Sorensen 2001).

Population genetic data for Pacific lampreys are scarce, and the small numbers of loci and limited geographical scope of these studies limit inferences about population structure and philopatry. Docker et al. (1999) found no differences (identical mitochondrial DNA sequences for the cytochrome *b* and NADH dehydrogenase subunit 3 genes) among Pacific lampreys from two different streams on Vancouver Island, British Columbia, and a river on the southern Oregon coast and no differences between Pacific lampreys and Vancouver lampreys *L. macrostoma*, Pit-Klamath brook lampreys *L. lethophaga*, or Klamath lampreys *L. similis* from California. The latter three species are nonanadromous and are considered to be derivatives of the Pacific lamprey (Hubbs 1971; Vladykov and Kott 1979; Beamish 1982). Likewise, Goodman (2006) found no significant geographical differences in mitochondrial DNA haplotypes among Pacific lampreys from British Columbia to California, although many southern samples had private haplotypes. Lorion et al. (2000), however, noted sequence variation at the cytochrome *b* locus between anadromous and nonanadromous Pacific lampreys in the Klamath River basin, Oregon, and among Pacific lampreys, Pit-Klamath brook lampreys, and Klamath lampreys. Likewise, Beamish and Withler (1986) documented significant differences at allozyme loci between two collections from the Fraser River on mainland British Columbia and nearby Vancouver Island.

The status of the Pacific lamprey across its range is likewise poorly understood, and the lack of information on population structure of the species further hinders interpretation and management responses to local changes in abundances. Lampreys are generally vulnerable to many of the same kinds of threats facing populations of Pacific salmon *Oncorhynchus* spp. (important hosts for parasitic Pacific lampreys), including anthropogenic barriers to migration, degradation of spawning and rearing areas, mortality from hydroelectric turbines, and nonindigenous predators (Renaud 1997). In areas of the Pacific Northwest where tallies of abundance are available, numbers of Pacific lampreys appear to have decreased exponentially in the last 50 years (Close et al. 2002).

In this study, we investigated the use of amplified fragment length polymorphisms (AFLPs) to assess genetic population structure of Pacific lampreys. The AFLP technique was developed for genomic DNA fingerprinting (Vos et al. 1995). Unlike many other techniques, AFLP has the potential to generate information from hundreds of loci quickly, precisely, and inexpensively, which makes it useful for studying organisms where other genetic markers show low levels of polymorphisms. Since AFLP markers are dominant, identifying homologous alleles is difficult and some kinds of population genetic analyses are limited (Mueller and Wolfenbarger 1999; Savelkoul et al. 1999). Amplified fragment length polymorphism markers have not been widely used for population genetic studies of fish. Most molecular systematic or population genetic studies of lamprey species have been recently carried out using allozymes (e.g., Wright et al. 1985; Engelhorn and Schreiber 1997; Yamazaki and Goto 1998; Yamazaki 2002), sequencing portions of the mitochondrial DNA (e.g., Tagliavini et al. 1994; Docker et al. 1999; Yamazaki et al. 2003), restriction fragment length polymorphism analysis of mitochondrial DNA sequences (Docker et al. 2007), microsatellite DNA (Bryan et al. 2005), and random amplified polymorphic DNA analysis by polymerase chain reaction (PCR; Mejia et al. 2004; Yamazaki et al. 2005). However, AFLP markers have been widely applied to study population differentiation, genetic diversity, and genetic architecture in a wide variety of organisms (primarily plants, bacteria, fungi, agricultural species, and agricultural pests; Bensch and Åkesson 2005), including black tiger shrimp *Penaeus monodon*, kuruma prawns *P. japonicus* (Wilson et al. 2002; Li et al. 2003), pollen beetles *Meligethes aeneus* (Kazachkova et al. 2004), yellow fever mosquitoes *Aedes aegypti* (Yan et al. 1999), and wild turkeys *Meleagris gallopavo* (Mock et al. 2002). Similarly, most AFLP applications for fish have focused on cultured species, such as Nile tilapia *Oreochromis niloticus*, channel catfish *Ictalurus punctatus*, and common carp *Cyprinus carpio* (Kocher et al. 1998; Liu et al. 1999; Wang et al. 2000). Despite the potential usefulness of AFLPs, only a few studies have used these markers to address ecological or evolutionary problems in natural populations of fish (e.g., Whitehead et al. 2003; Campbell and Bernatchez 2004; Han and Ely 2004; McMillan et al. 2006).

Methods

Samples.—Samples of migrating adult Pacific lampreys were collected from seven freshwater locations in North America and one in Asia (Figure 1). Thirty specimens were collected from each of the seven North American locations: (1) Moose River, Alaska; (2) North Fork Toutle River, Washington; (3) Willamette River Falls, Oregon; (4) Sherars Falls on the Deschutes River, Oregon; (5) John Day Dam on the Columbia

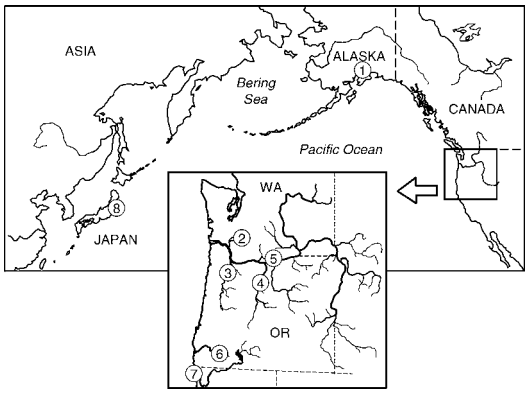

FIGURE 1.—Locations where Pacific lampreys were collected for a study of population genetic structure: (1) Moose River, Alaska; (2) North Fork Toutle River, Washington; (3) Willamette River Falls, Oregon; (4) Sherars Falls on the Deschutes River, Oregon; (5) John Day Dam on the Columbia River; (6) Savage Rapid Dam on the Rogue River, Oregon; (7) the mouth of the Klamath River, California; and (8) Naka River, Japan.

River; (6) Savage Rapid Dam on the Rogue River, Oregon; and (7) the mouth of the Klamath River, California. Muscle or fin tissues from each fish were stored in ethanol. Eight specimens were collected from Naka River, Japan.

Extraction of DNA and construction of amplified fragment length polymorphism fingerprint.—Genomic DNA was extracted from Pacific lamprey tissues with a DNeasy Blood and Tissue Kit (QIAGEN, Inc., Mississauga, Ontario). Structure AFLP fingerprint pattern, working technique, and response liquid are as described by Wang et al. (2002). Briefly, genomic DNA (200 ng) was double digested using two restriction enzymes (*Eco*R I and *Mse* I, 1.25 units/μL each) in a total volume of 25 μL. Subsequently, double-stranded adapters were ligated to the restricted fragments at 4°C overnight. Adapter sequences for *Eco*R I were 5′-CTCGTAGACTGCGTACC-3′ and 3′-CATCTGACGCATGGTTAA-5′. Sequences for *Mse* I were 5′-GACGTGAGTCCTGAG-3′ and 3′-TACTCAGGACTCAT-5′. The ligation products were preamplified using *Eco*R I + 0 and *Mse* I + C primers in a total volume of 50 μL containing 5 μL of ligation mixture (diluted 10 times in tris–EDTA [TE]). Sequences of *Eco*R I + 0 and *Mse* I + C preamplification primers were 5′-GACTGCGTACCAATTC-3′ and 5′-GATGAGTCCTGAGTAA-3′, respectively. Selective amplification was performed in a 50-μL final volume containing 5 μL of preamplified products (diluted 50 times in TE). The seven selective amplification primer combinations had an additional 2–3-nucleotide extensions at the 3′ end and were E-TT/M-CTC, E-TA/M-CAG, E-AC/M-CTG, E-TC/M-CTC, E-TT/M-CTG, E-TA/M-CTG, and E-TC/M-CTG. Preselective ′amplification was performed by 20 cycles of amplification (94°C for 30 s, 56°C for 60 s, and 72°C for 60 s). The diluted, preamplified products were used for further selective amplification in the following protocol: 1 cycle of 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s; 12 cycles of amplification with annealing temperature decreasing by 0.7°C in each cycle (starting at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s); and 23 cycles of amplification (94°C for 30 s, 56°C for 30 s, and 72°C for 60 s). The PCR products were mixed with equal proportions of a 98% solution of formamide, 10-mM EDTA, 0.05% bromphenol blue (tracking dye), and 0.05% xylene cyanol (tracking dye); these were denatured at 90°C for 3 min and quickly cooled on ice. Three-microliter aliquots of sample solution were loaded onto a denaturing 6% polyacrylamide gel in 1× tris–boric acid–EDTA buffer using a BioRad sequence gel system. After running, gels were silver stained to show amplified products.

Data analysis.—The AFLP bands were scored for presence or absence, and scores were transformed into a binary character matrix. Fragments that could not be scored unambiguously were not included in the analysis. We calculated number of amplified bands and number of polymorphic bands by the methods of Wang et al. (2002) and Pan et al. (2002). Percentage of polymorphic loci and Nei's (1973) unbiased expected gene diversity (heterozygosity) were calculated for each geographical sample using POPGENE software (Yeh et al. 1999). We examined patterns of genetic variation among samples by calculating Nei's genetic distance (D_a; Nei et al. 1983) after discarding bands that were monomorphic across all individuals to reduce statistical bias (Keiper and McConchie 2000). We constructed neighbor-joining dendrograms (Saitou and Nei 1987) with bootstrap values based on 5,000 repetitions using Genetic Distance and Phylogenetic Analysis (DISPAN) software. We tested for statistically significant variation among samples and groups by use of analysis of molecular variance (AMOVA) and calculated overall and pairwise genetic differentiation indices (F_{ST}) using Arlequin version 3.1 (Excoffier et al. 2005) with 1,000 permutations. Allele frequencies were based on Lynch and Milligan's (1994) expansion method. We tested AMOVAs using several possible scenarios of geographical organization. These were also used to guide Bayesian population clustering performed in a version of STRUCTURE software (Pritchard et al. 2000) modified to handle data for dominant genetic markers. We tested scenarios of 2–16 hypothetical clusters using a 50,000-repetition burn-in

TABLE 1.—Number of amplified bands and number (*N*) and percentage of polymorphic bands for seven primer combinations from 218 Pacific lampreys collected in Alaska, the Pacific Northwest, and Japan.

Primer combination	Samples scored	Bands amplified	Polymorphic bands	
			N	%
E-TT/M-CTC	218	72	24	33.3
E-AC/M-CTG	218	83	21	25.3
E-TT/M-CTG	218	81	32	39.5
E-TC/M-CTC	218	85	32	37.6
E-TA/M-CAG	218	79	32	40.5
E-TA/M-CTG	218	71	9	12.7
E-TC/M-CTG	218	85	30	35.3
Total	1,526	556	180	32.4

period and 950,000 repetitions. We tested for isolation by distance using Mantel tests for correlation between matrices of pairwise geographical distance and F_{ST} and by reduced-axis regression (Sokal and Rolf 1981) in Isolation by Distance computational software (Bohonak 2002).

Results

Amplified fragment length polymorphism analysis of 218 individuals based on seven primer combinations resolved a total of 556 interpretable bands. Segments less than 50 base pairs were difficult to score and were not used. Across all samples, 180 (32.4%) bands were polymorphic. The percentage of polymorphic bands for each pair of primer combinations ranged from 12.7% to 40.5%. The greatest number of amplified fragments was produced by E-TC/M-CTG and E-TC/M-CTC, and the smallest number was produced by E-TA/M-CTG. The largest proportion of polymorphic bands was produced by E-TA/M-CAG, although E-TT/M-CTG and E-TC/M-CTC produced an equal number (Table 1).

Across the range of the species, Pacific lamprey collections in this study were characterized by relatively low polymorphism and a large number of shared bands. The percentage of polymorphic bands ranged from 26.1% in the Deschutes River collection to 30.1% in the Naka River collection. Less-common bands (those with a frequency of $< 50\%$) made up only a small proportion of the polymorphic bands, ranging from 2.6% in the John Day River to 5.4% in the Naka River. No collections had unique bands. Assuming each band represented a gene locus, expected heterozygosity over all loci ranged from 0.066 in the Deschutes River to 0.111 in the Naka River (Table 2); these were the only statistically different heterozygosity values ($P < 0.05$).

Significant differences existed in AFLP variation among collections. The AMOVA testing for differences across three geographical regions—the Pacific Northwest, Alaska, and Japan—showed an overall divergence among samples ($F_{ST} = 0.106$, $P < 0.001$) and significant differences among regions (regional differentiation index $F_{RT} = 0.014$, $P < 0.001$), among collections within the Pacific Northwest (among-population differentiation index $F_{SR} = 0.092$, $P < 0.001$), and within populations (Table 3). Examination of the data suggested that collections from the Naka and Deschutes rivers were the most divergent, and the treatment of the Naka River, Deschutes River, and all remaining samples as three groups in the AMOVA produced the largest partitioning of genetic variation among groups for all analyzed scenarios (among-group differentiation index $F_{GT} = 0.062$, $P = 0.043$; $F_{ST} = 0.134$, $P < 0.001$). Pairwise F_{ST} values between the collections ranged from 0.037 between the Willamette and North Fork Toutle rivers (two geographically proximate locations separated by 148 km in the lower Columbia River) to 0.256 between the Deschutes and Naka rivers (locations separated by over 8,000 km; Table 4). All pairwise F_{ST} comparisons were statistically significant ($P < 0.001$).

Pacific lampreys showed a geographical pattern of divergence across the range of the species but no clear pattern of geographical structure within the Pacific

TABLE 2.—Amplified fragment length polymorphism variation in 218 Pacific lampreys from eight collections throughout the species' range (see Figure 1 for collection locations); total number of amplified bands, number (*N*) and percentage of polymorphic and less-common bands, and expected heterozygosity (H_e) are shown. Private bands were not found in any sample.

Collection site	Amplified bands	Polymorphic bands		Less-common bands		H_e
		N	%	*N*	%	
Moose River	545	159	29.2	7	4.4	0.084
North Fork Toutle River	544	154	28.3	6	3.9	0.082
Willamette River	548	164	29.9	5	3.0	0.087
Deschutes River	528	138	26.1	5	3.6	0.066
John Day Dam	541	152	28.1	4	2.6	0.075
Rogue River	547	161	29.4	6	3.7	0.083
Klamath River	546	158	28.9	5	3.2	0.082
Naka River	553	167	30.2	9	5.4	0.111

TABLE 3.—Results of analysis of molecular variance in Pacific lampreys from eight collections (Figure 1) representing three geographical regions (Alaska, the Pacific Northwest, and Japan). All sources of variation were significant ($P < 0.001$) based on 1,000 permutations (df = degrees of freedom; SS = sum of squares).

Source of variation	df	SS	Variance components	Percent of variance
Among regions	2	169.4	0.378	1.4
Among populations within regions	5	493.806	2.485	9.2
Within populations	210	5,084.625	24.213	89.4

Northwest. Bootstrap values from the neighboring-joining dendrogram showed strong support for divergence of Pacific lampreys in Japan and Alaska from those in the Pacific Northwest, and the Japanese collection was the most divergent (Figure 2). Within the Pacific Northwest, the dendrogram showed weak support for a lower Columbia River cluster comprised of the North Fork Toutle and Willamette rivers and strong support for the Deschutes River sample as an outlier. Samples from the Rogue and Klamath rivers, which are in the Klamath Mountain Province, were not distinct from Columbia River samples in the cluster analysis. Likewise, the AMOVA testing for differences between two Pacific Northwest biogeographical regions—the Columbia River and Klamath Mountain Province (Figure 1)—showed no significant differences. Across the range of the species, Pacific lampreys showed a weak but statistically nonsignificant tendency for decreasing gene flow with increased geographical distance (Figure 3). When we excluded Deschutes River samples from the analysis as outliers because they showed strong isolation from all other samples, significant isolation by distance was detected across the entire range ($Z = 7{,}820.5$, $r = 0.71$, $P = 0.015$) but not within the Pacific Northwest ($Z = 781.0$, $P = 0.652$). Genetic exchange between the Pacific Northwest and Naka River, as estimated from F_{ST} values, was approximately 2 migrants/generation, whereas exchange among Pacific Northwest locations ranged from 1 migrant/generation to almost 7 migrants/generation (Figure 3).

Analysis of the data using STRUCTURE to assign genotypes to lineages suggested at least six hypothetical aggregations (Table 5). STRUCTURE analysis of more than six aggregations (number of clusters $K = 7$–16) showed similar patterns and only slightly lower likelihoods. We report the results for six aggregations because they are the easiest to interpret, but the patterns held for all analyses. Most of the hypothetical aggregations corresponded to locations where Pacific lampreys were collected (Table 5; Figure 4). Pacific lampreys from the Moose River collection mostly occurred in the same hypothetical groups (cluster 1). Pacific lampreys from the Rogue River collection were also assigned to the same groups (cluster 2), as were some Pacific lampreys from the Klamath River, a coastal river that is situated to the south of the Rogue River. Pacific lampreys from the Deschutes River collection predominated in cluster 3. Likewise, Pacific lampreys from the two lower Columbia River collections, the Willamette and North Fork Toutle rivers, consistently were assigned to the same groups (cluster 5).

Several patterns were unexpected. Pacific lampreys from the Naka River consistently were assigned to two different clusters, such as clusters 1 and 4. In contrast, Pacific lampreys that were collected several hundred kilometers upstream in the Columbia River at the John Day Dam were consistently grouped with fish from the Klamath River collection on the northern California coast. However, in general, Klamath River Pacific lampreys, which were collected at the mouth of the river as they migrated from the Pacific Ocean, were the least likely to be assigned to a single group.

Discussion

Our results suggested considerable historical gene flow over the range of Pacific lampreys and a stepping

TABLE 4.—Pairwise values of Nei's genetic distance (D_a; above diagonal) and the genetic differentiation index (F_{ST}; below diagonal) for Pacific lampreys from eight collections (Figure 1). All F_{ST} comparisons were significant ($P < 0.001$) based on 1,000 permutations.

Collection site	Collection number							
	1	2	3	4	5	6	7	8
Moose River	0	0.0123	0.0092	0.0180	0.0092	0.0147	0.0086	0.0107
North Fork Toutle River	0.110	0	0.0098	0.0218	0.0164	0.0118	0.0083	0.0204
Willamette River	0.075	0.037	0	0.0202	0.0111	0.0139	0.0073	0.0163
Deschutes River	0.167	0.182	0.151	0	0.0168	0.0129	0.0180	0.0298
John Day Dam	0.072	0.130	0.071	0.118	0	0.0149	0.0075	0.0176
Rogue River	0.126	0.078	0.084	0.098	0.101	0	0.0102	0.0216
Klamath River	0.072	0.069	0.050	0.138	0.042	0.063	0	0.0167
Naka River	0.070	0.148	0.128	0.256	0.148	0.142	0.118	0

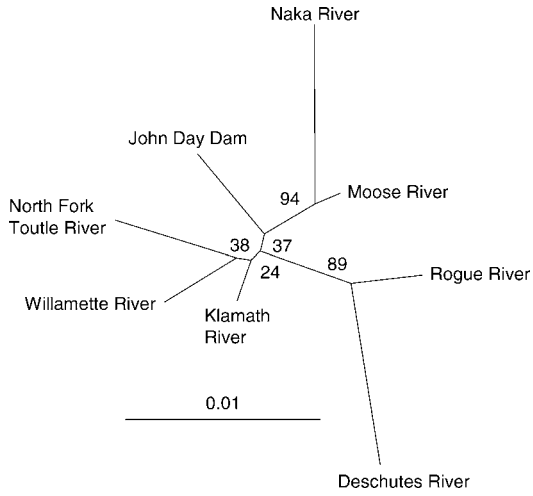

FIGURE 2.—Unrooted neighbor-joining dendrogram based on Nei's genetic distance (D_a), illustrating genetic relationships between Pacific lamprey collections from Alaska, the Pacific Northwest, and Japan (Figure 1). Numbers at nodes show bootstrap percentage values greater than 50% based on 5,000 repetitions.

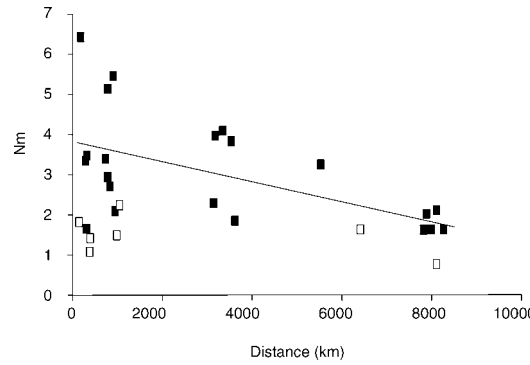

FIGURE 3.—Relationship between the genetic differentiation index (F_{ST}) and geographical distance separating Pacific lamprey collections from Alaska, the Pacific Northwest, and Japan (see Figure 1; black diamonds = comparisons with the Deschutes River, Oregon, collection; white squares = all other comparisons). The dotted regression line describes the relationship for all comparisons; the solid regression line describes all but the Deschutes River comparisons.

stone pattern of exchange that allowed genetic differentiation of Pacific lampreys occurring in major geographical regions. However, this pattern did not hold for Pacific lampreys within the Pacific Northwest, where we documented significant genetic differences among fish from different locations but found no obvious geographical pattern of gene flow or differentiation. We do not yet have a good explanation for this disparity, but it calls attention to the need for better data and analyses of the demographic status, genetic variation, and migratory behavior of Pacific lampreys.

The high frequency of shared alleles in all samples from northern California, Alaska, and Japan is evidence of persistent historical gene flow. Almost all alleles were shared among these collections, although the dominant nature of AFLP data makes it difficult to identify homologous alleles (Mueller and Wolfenbarger 1999). We observed no private bands in any samples. Similarly, the occurrence of less-common bands (those with an overall frequency of $<50\%$) was rare, ranging from 2.6% to 5.4% over 138–167 polymorphic loci (Table 2).

Maintaining this allelic diversity would have been possible with a stepping stone pattern of gene flow along the North American and Asian coastlines. Our AMOVA showed significant differences among collections of Pacific lampreys from the Pacific Northwest, Alaska, and Japan (Table 3). When the Deschutes River collection was treated as an outlier, we found a strong pattern of decreasing gene flow and greater genetic differentiation with increasing geographical distance (Figure 3), which are characteristic of stepping stone gene flow patterns (Slatkin 1993). Our analyses indicated that genetic differences between collections from the Naka and Moose rivers, for example, could be explained by exchange of approximately three genetically effective migrants per generation (Figure 3). This level of exchange is large enough to prevent fixation despite the geographical separation of the two spawning areas by over 5,000 km, and yet it allows significant divergence in allele frequencies (Wright 1931; Spieth 1974; Allendorf and Phelps 1981).

This estimate of genetic exchange is unlikely to reflect direct demographic exchange of numbers of individuals, however (Mills and Allendorf 1996). Pacific lampreys are distributed along the North Pacific rim from California through the Aleutian Islands, and in Asia as far as Japan (Scott and Crossman 1973). This distribution provides an avenue for demographic exchange over shorter geographical distances that leads to gene flow over larger geographical distances. Pacific lampreys from one river system may mix in the ocean with those from other river systems and subsequently return as spawners to interbreed with other populations nearby or even hundreds of kilometers up or down the coastline, but mixing of populations does not necessarily occur across the range of the species. Over generations, alleles can move along the coast via a stepping stone pattern and can spread across large geographical areas. Compared with sea lampreys, which are also separated by two continents but which have diverged to the extent that they are fixed for

TABLE 5.—Bayesian assignment (proportion of gene pool assigned) of 218 Pacific lampreys from eight collections (Figure 1) to six hypothetical genetic lineages based on analysis with STRUCTURE software.

Collection site	Sample size	Lineage					
		1	2	3	4	5	6
Moose River	30	0.545	0.020	0.031	0.143	0.059	0.203
North Fork Toutle River	30	0.069	0.064	0.028	0.049	0.758	0.032
Willamette River	30	0.121	0.047	0.039	0.117	0.504	0.172
Deschutes River	30	0.016	0.025	0.885	0.030	0.015	0.029
John Day Dam	30	0.082	0.029	0.068	0.121	0.019	0.681
Rogue River	30	0.036	0.632	0.119	0.061	0.095	0.057
Klamath River	30	0.158	0.159	0.029	0.065	0.165	0.423
Naka River	8	0.465	0.022	0.007	0.473	0.017	0.016

different mitochondrial DNA haplotypes (Rodríguez-Muñoz et al. 2004), gene flow along the geographical distribution of Pacific lampreys appears to have prevented fixation of different alleles between fish from the two continents.

The life history of Pacific lampreys may contribute to gene flow. During the 6–40-month ocean feeding phase, for example, Pacific lampreys migrate into waters generally greater than 70 m deep and up to 40 km offshore and attach to their hosts, which are often

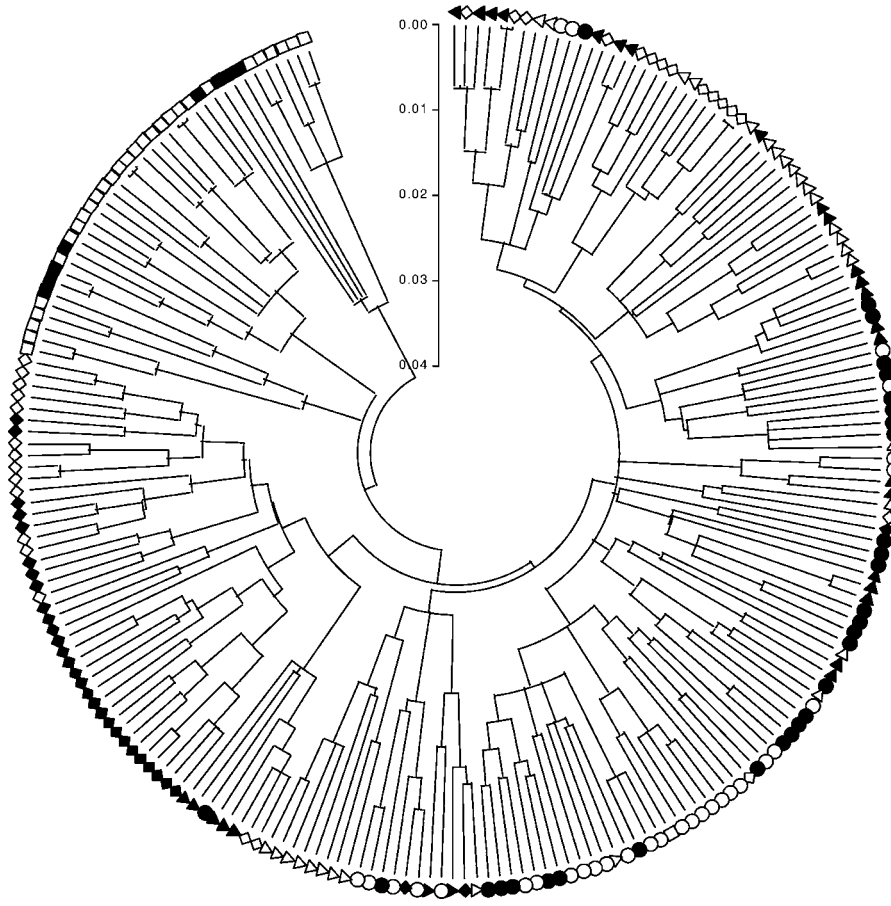

FIGURE 4.—Probabilities of Pacific lamprey assignment ($n = 218$ genotypes) to six hypothetical aggregations (columns 1–6) based on analysis with STRUCTURE software. Horizontal dashed lines delineate collection locations (see Figure 1; NF = North Fork).

migratory, for up to several days (Kan 1975; Beamish 1980). The distances that Pacific lampreys migrate or are carried by currents and their prey are unknown, although catches in the Bering Sea suggest that these fish travel hundreds of miles (cited in Beamish 1980). This method of dispersal may be somewhat opportunistic and could lead to variable dispersal patterns depending on location, ocean entry time, and variation in oceanographic processes, but it would allow Pacific lampreys to follow coastlines and mix with populations from other rivers.

Within the Pacific Northwest, the AMOVA and pairwise F_{ST} comparisons showed significant differences among Pacific lampreys from different locations (Tables 3, 4). These differences did not follow a pattern of isolation by distance. With the exception of weak evidence for a lower Columbia River aggregation consisting of Willamette and North Fork Toutle River populations (Figure 2; Table 5), genetic differences did not correspond to any obvious biogeographic pattern that might suggest historical or recent shared patterns of gene flow. The similarity of Willamette and North Fork Toutle River collections, however, probably reflects recent recolonization of the North Fork Toutle River by Pacific lampreys from the other streams in the lower Columbia River, as well as straying of North Fork Toutle River adults that were in the ocean when Mount St. Helens erupted in 1980. This eruption introduced massive amounts of ash and volcanic debris into the North Fork Toutle River and surrounding basins, virtually devastating stream and riparian communities and upland forests. The importance of straying (i.e., by fish that survived because they were in the ocean and other refugia) in recolonization of this watershed after the eruption has also been documented for other species (Leider 1989; Hawkins and Sedell 1990). It was probably important for Pacific lampreys as well. Similarly, our collection at the John Day Dam on the Columbia River probably represents adults that would have spawned primarily in the John Day River because it is the main known natal area for Pacific lampreys above the dam (Moser and Close 2003). However, it could have included fish bound for other rivers.

In contrast, the divergence of the Deschutes River collection from other collections is puzzling. Sampling error or genetic drift of a local population appears unlikely, although we cannot dismiss the founder effect as an explanation. Given the range of observed F_{ST} values and number of polymorphic loci in our study, our sample size of 30 fish should accurately describe genetic differences among aggregations (Nei 1987; Kalinowski 2005). Furthermore, our collections were of migrating adult Pacific lampreys, which minimized error associated with sampling ammocoetes. Fecundity of Pacific lampreys can range from 15,000–240,000 eggs (Pletcher 1963; Kan 1975), and sampling of ammocoetes can result in genetic characterization of individuals from only a few families (Jacobson et al. 1984; Jacobson 1986). Estimates of polymorphism for the Deschutes River collection were lower than those for all other collections (which might suggest local genetic drift), but the estimates were not statistically different from other collections except the Naka River sample, which had an expected heterozygosity almost twice as great (Table 2). Counts of migrating adults returning to the Deschutes River number in the thousands (authors' unpublished data), which does not immediately suggest low effective population size (N_e). Demographic characteristics that affect the relationship between census size and N_e for Pacific lampreys, however, are mostly unknown. Consequently, genetic drift cannot be ruled out entirely.

An alternative explanation is that the high fecundity of Pacific lampreys—combined with high variance in reproductive success; an opportunistic, "sweepstakes" method of ocean dispersal; and imprecise natal homing—could lead to temporally unstable genetic differences among adult spawners. From year to year, these genetic differences among adult spawners may be larger over smaller geographical scales than persistent genetic differences occurring over larger geographical regions. Such a pattern might well look like that depicted in Figure 3. Although unusual for freshwater spawning fishes, an analogous explanation has been proposed to explain similar genetic patterns in some widely distributed marine species that exhibit sweepstakes-type reproductive success (Hedgecock 1994a, 1994b). Information on temporal genetic variation in Pacific lampreys over a larger geographical area—combined with better information on demographic characteristics of spawners, dispersal patterns in the ocean, and adult migration mechanisms—will help test this hypothesis.

Our results are among the first to suggest genetic structure in Pacific lampreys. Although Beamish and Withler (1986) documented significant differences at allozyme loci between two collections from the Fraser River and nearby Vancouver Island, their study was not focused on genetic population structure of Pacific lampreys, and their limited analysis of variation has not been widely cited. In the absence of genetic and demographic data, many biologists have presumed that adult migratory behavior and natal philopatry in Pacific lampreys are similar to those in sea lampreys, which have a similar life history but occur in Atlantic Ocean watersheds. Anadromous sea lampreys in North America appear to be panmictic (Bryan et al. 2005),

although they have diverged from European populations (Rodríguez-Muñoz et al. 2004). Likewise, the pouched lamprey *Geotria australis*, a species found in the southern hemisphere, apparently interbreeds over distances of several thousand kilometers (Johnston et al. 1987). Bergstedt and Seelye (1995) found no evidence of natal stream fidelity in sea lampreys from Lake Huron based on a tagging study of juveniles. Instead, migrating adult sea lampreys chose spawning locations based on the presence of ammocoetes, which produce petromyzonol sulfate and allocholic acid (bile acids that are detected by the adults; Bjerselius et al. 2000; Vrieze and Sorensen 2001). Petromyzonol sulfate has also been identified in other lamprey species, including the Pacific lamprey (Yun et al. 2003; Fine et al. 2004), which suggests that it is used by Pacific lampreys also.

Our results appear to differ from those of Goodman (2006), who found no evidence of mitochondrial DNA divergence in 81 collections of Pacific lampreys from British Columbia to California. Within the Pacific Northwest, we found no significant difference between Pacific lampreys from two of the geographical regions common to the two studies (Columbia River and Klamath Mountain Province); Goodman (2006) also found no difference between collections from these regions. Unlike Goodman (2006), however, we demonstrated significant differences among collections within those regions. The differences between these studies may reflect the increased power of using approximately 180 AFLP loci versus a single mitochondrial DNA locus or differences in polymorphisms due to sampling of adult migrants versus ammocoetes; the differences could also be explained by female-mediated gene flow (Prugnolle and de Meeus 2002). The latter seems unlikely based on current knowledge, however, because Pacific lampreys tend to be polygynous (Pletcher 1963), and current hypotheses for sex-biased dispersal predict male-biased dispersal in polygynous species (Favre et al. 1997).

Our results suggest that conservation efforts to understand and stop the downward trend in abundance and distribution of Pacific lampreys must consider geographical genetic differences within the species. Although we detected significant genetic differences among adult Pacific lampreys returning to streams separated by as little as 87 km (between the Deschutes River and John Day Dam), the geographical scale over which genetically meaningful management units (e.g., stocks, populations, or evolutionarily significant units) occur in this species cannot be identified based on our data. This will require more expansive geographical and temporal sampling than was possible from our limited study design. Given only weak evidence that genetic differences correspond to geographical patterns in the Pacific Northwest, it is especially important to determine the scale at which these patterns are temporally stable. Future genetic studies using an adequate number of polymorphic loci and physiological and demographic studies of Pacific lamprey migration will almost certainly resolve this question.

Acknowledgments

We would like to thank the Bonneville Power Administration and New Century Millions of Talents Program in China for funding this work, and we are grateful to the Warm Springs and Yurok tribes and the Alaska Department of Fish and Wildlife for providing samples.

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North American Journal of Fisheries Management 28:1648, 2008

DOI: 10.1577/M07-035.E

ERRATUM

Please make the following correction in a recent issue of this journal.

Volume 28(4), August 2008: "Amplified Fragment Length Polymorphism Assessment of Genetic Diversity in Pacific Lampreys" by Binbin Lin, Ziping Zhang, Yilei Wang, Kenneth P. Currens, Adrian Spidle, Yuji Yamazaki, and David A. Close, pages 1182–1193.

Page 1188. Please replace Figure 4 with the correct Figure 4 (below).