Microsatellites Reveal Population Identity of Individual Pink Salmon to Allow Supportive Breeding of a Population at Risk of Extinction

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Abstract.—Efforts to restore depressed populations of Pacific salmon *Oncorhynchus* spp. are often hampered by the inability to assign population identity to individuals in an admixture. This knowledge is of particular concern in supportive breeding programs, in which misidentification of individuals to population may result in progeny of mixed heritage, which, in turn, results in the erosion of the genetic population structure and of the existing genetic diversity and local adaptations of the target population. We evaluated two classes of genetic markers, allozymes and microsatellites, for estimating population identity of pink salmon *Oncorhynchus gorbuscha* in a supportive breeding program on the Dungeness River in Washington State. Fall-run pink salmon of the Dungeness River are the target of restoration, but they presumably overlap, in terms of timing, with an earlier summer run. Both marker types revealed similarly low estimates of relative genetic differentiation ($\hat{\theta} = 0.02$), which suggests that there is little variation in allele frequency among populations. However, microsatellites provided a more accurate estimate of population identity. When applying a log-likelihood ratio criterion of greater than 1.3, 74.8% of individuals were correctly assigned to population using microsatellites (versus 3.1% of individuals using allozymes). The difference in assignment accuracy was best predicted by the statistic $\hat{\delta}$, which estimates cumulative allele frequency differences among populations. Our results suggest that genetic markers with many alleles are preferred when populations exhibit little genetic differentiation (as is the case in pink salmon), because $\hat{\delta}$ is more likely to be large, presumably as a result of genetic drift at each allele. The use of microsatellites to select fall-run pink salmon for supportive breeding confirmed the run-timing overlap and prevented unintentional crosses between the two populations.

When natural populations are at risk of extinction or critical loss of genetic diversity, one management option is supportive breeding (Ryman et al. 1994). In order to improve survival, wild parents representing a fraction of the natural population are bred in captivity and their progeny are released, after some captive rearing, into natural habitats. Supportive breeding is often considered for restoration of populations of Pacific salmon *Oncorhynchus* spp. because culture techniques and systems are well developed. Examples include endangered Snake River sockeye salmon *Oncorhynchus nerka* (Cummings et al. 1997) and Sacramento River winter run chinook salmon *O. tshawytscha* (Hedrick et al. 1994).

If a population targeted for supportive breeding mingles with one or more other populations when founders are selected, identification error becomes a risk. Misidentification of individuals to population may result in progeny of mixed heritage, which erodes the genetic population structure and the existing genetic diversity and local adaptations of the target population (Waples 1994). Without a method of determining population affiliation, resource managers should consider alternatives to supportive breeding in order to maintain genetic diversity.

In the absence of reliable phenotypic identifiers,

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molecular genetic markers offer the only approach to identifying individuals to population. The most useful markers for estimating population identity are those with large allele frequency differences among populations. Shriver et al. (1997) called such alleles ''population specific alleles'' (PSAs), and they defined a large frequency differential as one of greater than 50%. In the absence of selection, the allele frequency differential varies randomly among loci and is a function of genetic drift. Thus, high heterozygosity and large numbers of alleles are not necessarily indicative of the usefulness in terms of estimating population affiliation. Marker types that tend to be less polymorphic (e.g., allozymes) may be as useful as more variable markers (e.g., microsatellites).

In their native range, pink salmon *O. gorbuscha* are anadromous and semelparous, and they exhibit a rigid 2-year life cycle that has resulted in two reproductively isolated lineages called odd- and even-year lineages (Davidson 1934; Heard 1991). Puget Sound and the Strait of Juan de Fuca in Washington State are the southern limit of persistent spawning populations of pink salmon (Hard et al. 1996). Of the 14 recognized stocks (1 even- and 13 odd-year stocks) in this region, two are of special interest—the odd-year summer and fall runs of the Dungeness River in the Strait of Juan de Fuca. They represent the southwestern boundary of the species range and are widely separated from other pink salmon populations in Puget Sound (Shaklee et al. 1995). Their geographic location, unique life history, and genetic variation contribute significantly to the ecological and genetic diversity of pink salmon in this region (Hard et al. 1996). Since 1979, the fall run has experienced a rapid decline in abundance, and since 1987, the number of potential breeders has averaged fewer than 400 individuals (Washington Department of Fish and Wildlife, unpublished data). In contrast, the summer-run population has averaged over 6,000 potential breeders for the same period. Recent reviews have described the status of the fall run as ''critical'' (Washington Department of Fisheries 1993), at ''moderate risk of extinction'' (Nehlsen et al. 1991), and ''at risk'' (Hard et al. 1996). The drastic decline of Dungeness River fall pink salmon prompted resource managers to propose a supportive breeding program in 1997.

Stream survey data show that the summer and fall populations are spatially isolated during spawning (Figure 1). However, run-timing data did not rule out the possibility of some temporal overlap during river entry, particularly in mid-August (Figure 1). Because the fall-run population spawns near the river mouth, some interception of upriver-migrating, late summer-run fish could occur when selecting fall-run broodstock in August. A method was needed to distinguish fall- from summer-run pink salmon.

With the exception of Estoup et al. (1998), few studies have examined molecular markers for assigning population identity in fishes. Therefore, this study was begun to evaluate molecular methods for assigning population identity in pink salmon. Two objectives were defined. The first was to evaluate the utility of two marker types (allozymes and microsatellites) for determining population structure and population identity of Dungeness River summer and fall pink salmon. These nuclear DNA markers were chosen because laboratory protocols are well described and because they represent a range of polymorphism, a range that extends from relatively low (allozymes) to high (microsatellites) polymorphism. The second objective was to use molecular markers for selecting fall-run pink salmon for broodstock from the 1997 return. The results of this study were compared to those of Estoup et al. (1998) and to those of similar studies of other organisms.

Methods

Sample collection.—In 1995, tissue samples were collected from 100 summer-run and 62 fall-run Dungeness River pink salmon after spawning. Selection of individuals for each population was based on location of capture (Figure 1). Samples from heart, liver, muscle, and eye were collected and stored at $-80°C$ for allozyme analysis by Washington Department of Fish and Wildlife personnel. Samples of fin were collected for microsatellite analysis and were preserved in 100% ethanol.

In 1997, fin tissue for microsatellite analysis was collected from 171 prespawning adults (offspring of adults from the 1995 return) at a weir that was 0.6 km from the mouth of the river (Figure 1). Sampling began August 13, 1997, and ended September 17, 1997. All fish were given a single external tag and were transferred to Hurd Creek Hatchery, where they were held until genotyping was complete and a determination of population identity was made.

Allozyme analysis.—Alleles for 25 polymorphic allozyme loci were resolved using horizontal starch gel electrophoresis and enzyme-specific histochemical staining procedures de-
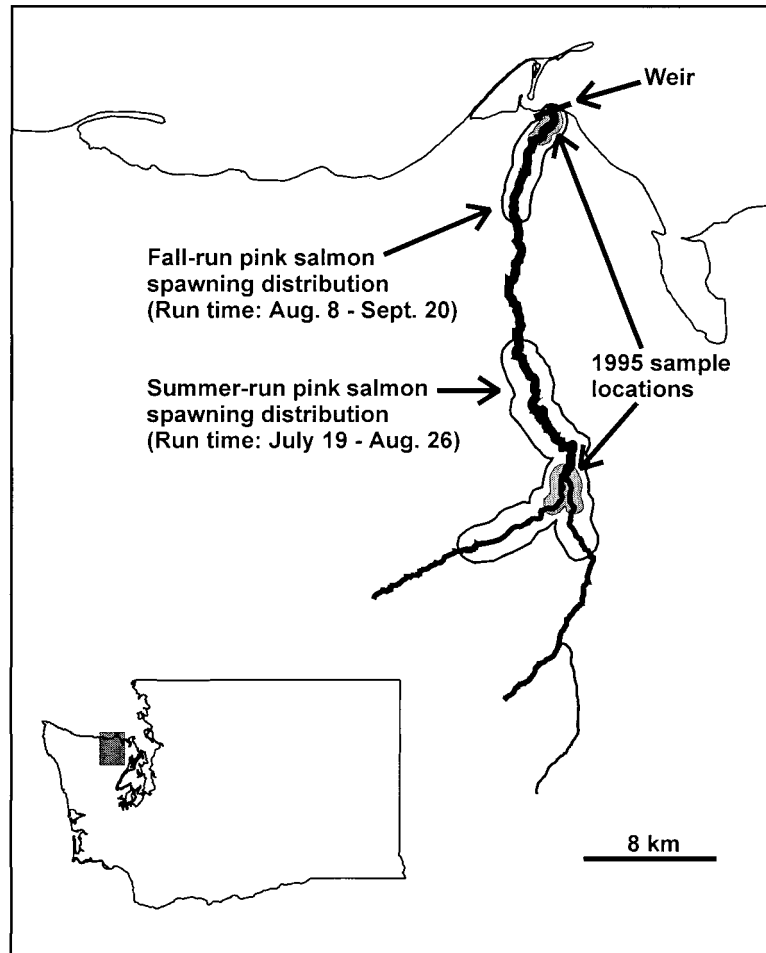
FIGURE 1.—Map of the Dungeness River and Washington State (inset). Approximate run timing, spawning distribution, and sample locations are shown for summer- and fall-run pink salmon.

scribed by Aebersold et al. (1987) and Shaklee et al. (1991). Enzyme Commission (EC) numbers are those of IUBMBNC (1992). The enzymes and their loci were aspartate aminotransferase, EC 2.6.1.1 (*mAAT-1**, *sAAT-3**, *sAAT-4**); adenosine deaminase, EC 3.5.4.4 (*ADA-2**); aconitate hydratase, EC 4.2.1.3 (*mAH-4**, *sAH**); alanine aminotransferase, EC 2.6.1.2 (*ALAT**); creatine kinase, EC 2.7.3.2 (*CK-A1**, *CK-C1**, *CK-C2**); formaldehyde dehydrogenase, EC 1.2.1.46 (*FDHG**); guanine deaminase, EC 3.5.4.3 (*GDA**); glucose-6-phosphate isomerase, EC 5.3.1.9 (*GPI-B2**); glycerol-3-phosphate dehydrogenase, EC 1.1.1.8 (*G3PDH-1**, *G3PDH-2**); isocitrate dehydrogenase, EC 1.1.1.42 (*sIDHP-2C**); L-lactate dehydrogenase, EC 1.1.1.27 (*LDH-A1**, *LDH-B1**); malate

dehydrogenase, EC 1.1.1.37 (*sMDH-B1,2**); mannose-6-phosphate isomerase, EC 5.3.1.8 (*MPI**); proline dipeptidase, EC 3.4.13.9 (*PEPD-2**); peptidase-LT, EC 3.4.-.- (*PEP-LT**); phosphogluconate dehydrogenase, EC 1.1.1.44 (*PGDH**); phosphoglucomutase (*PGM-2**).

Microsatellite analysis.—Total genomic DNA was isolated from 20–30 mg of fin tissue using a Gentra Systems (Minneapolis, Minnesota) Puregene DNA isolation kit. Precipitated DNA was hydrated in tris–ethylenediaminetetraacetic acid (EDTA) buffer (10 mM tris, 0.1 mM EDTA, pH 8.0) and heated at 55°C for approximately 12 h. The DNA concentration was measured by spectrophotometry, and then the extracts were diluted to 100 ng/μL for use in the polymerase chain reaction.

Seven microsatellite loci were scored using multiplexing procedures described by Olsen et al. (1996). The loci were μ*Sat60-1,* μ*Sat60-2* (Estoup et al. 1993), *Ots1* (Banks et al. 1999), *Ogo1a, Ogo3, Ogo4* (Olsen et al. 1998a), and *Ots101* (Small et al. 1998). Primers for the locus μ*Sat60* amplified two loci in pink salmon that differed in size by approximately 100 base pairs. The amplification of duplicate microsatellite loci has been reported in brown trout *Salmo trutta* (Estoup et al. 1998) and is not unexpected for salmonids, given their tetraploid ancestry (Allendorf and Thorgaard 1984). The allelic range of the μ*Sat60* duplicate loci did not overlap, and the loci exhibited disomic inheritance; therefore, each locus was treated as independent and was numbered using the protocol of Shaklee et al. (1990; e.g., μ*Sat60-1,* μ*Sat60-2*).

Microsatellites were size fractionated using an Applied Biosystems, Inc. (ABI) 373A automated DNA sequencer operated in GeneScan mode (ABI 1993). Data were analyzed using the internal lane sizing standard and local Southern sizing algorithm in the GeneScan 672 software version 1.1 (ABI 1993). Scoring of alleles for each locus and tabulation of data for importation into statistical software was performed with Genotyper software, version 2.0 (ABI 1996).

Statistical analysis.—Estimates of expected heterozygosity ($\hat{H}_E$) were calculated for allozyme and microsatellite loci using equation 8.4 of Nei (1987). The average $\hat{H}_E$ was calculated as the sum of $\hat{H}_E$ across loci divided by the number of loci. Tests for conformity to Hardy-Weinberg expectation (HWE) and genotypic linkage disequilibrium analyses were performed using a probability test in GENEPOP, version 3.1b (Raymond and Rousset 1995). Statistical significance levels (α) for the probability tests were determined using sequential Bonferroni adjustments for simultaneous tests (Rice 1989).

Unbiased estimates of Wright's *F* statistics (F_{IS} F_{ST} F_{IT}) were computed using the statistics $\hat{f}$, $\hat{\theta}$, and $\hat{F}$ (Weir and Cockerham 1984). Each statistic was computed for each locus and for all loci using FSTAT, version 1.2 (Goudet 1995). The probability that each statistic was greater than zero was tested in FSTAT by permuting the data set 10,000 times. Alleles were permuted within samples (individuals) to test $\hat{f}$ and among samples (individuals) to test $\hat{\theta}$ and $\hat{F}$.

Estimates of the allele frequency differential between populations were calculated for each locus using the following formula of Shriver et al. (1997):

$$\hat{\delta} = \left(\sum_{k=1}^{n} |F_k - S_k| \right) \Big/ 2.$$

In this formula, F_k and S_k are the frequencies of the *k*th allele in the fall and summer populations, respectively.

An estimate of population identity (fall or summer run) was made for each individual using the computer program WHICHRUN (Banks and Eichert 1999). Likelihood estimates for run origin were evaluated by calculating expected multilocus genotypic frequencies for test sample genotypes using observed allele distributions from either fall- or summer-run samples taken in 1995. This procedure assumes random mating and linkage equilibrium within populations and that both fall and summer populations are equally probable sources. Single locus genotype frequencies were computed as p_i^2 (homozygotes) and $2p_iq_i$ (heterozygotes), where *p* and *q* are allele frequencies at locus *i*. If an allele observed in test samples was missing from the baseline database of one or both populations, then a hypothesized frequency of $1/(2n + 1)$ was applied, where *n* is the number of samples in each baseline database. This conservative correction assumed that the allele would be the next allele sampled. Log-likelihood ratios (LLRs) of multilocus genotype frequencies were computed for each individual using the following formulas of Shriver et al. (1997):

$$\text{LLR}_{\text{AFS}} = \log_{10} \left(\prod_{i=1}^{n} Fi \Big/ \prod_{i=1}^{n} Si \right),$$

and

$$\text{LLR}_{\text{ASF}} = \log_{10} \left(\prod_{i=1}^{n} Si \Big/ \prod_{i=1}^{n} Fi \right).$$

In these formulas, *n* is the number of loci, and *Fi* and *Si* are the frequencies of individual A's genotype at locus *i* in the fall and summer populations, respectively. The usefulness of each marker type for population assignment was estimated by calculating the number of correct assignments at four LLR criteria: LLR>0, LLR>1.3, LLR>2, and LLR>3.

Two approaches were used to establish an LLR selection criterion for fall-run broodstock in 1997. First, only individuals from the 1995 baseline file were used in the test sample. These fish were called ''baseline.'' Second, 35 of the 100 summer-run individuals were removed from the microsatellite baseline to create a true unknown test sample with

TABLE 1.—Sample size (n), number of alleles per locus (A), expected heterozygosity ($\hat{H}_E$), $\hat{\theta}$, and $\hat{\delta}$ for microsatellites and allozymes. Values of $\hat{\theta}$ significantly greater than zero are marked with an asterisk; P-values were determined using a Bonferroni adjustment for multiple tests.

Locus	Sample size		A	$\hat{H}_E$		$\hat{\theta}$	$\hat{\delta}$
	Fall	Sum		Fall	Sum		
Microsatellites							
Ots1	58	91	14	0.851	0.877	*0.011	0.248
Ots101	57	89	32	0.915	0.876	0.007	0.375
μ*Sat60-1*	58	93	4	0.495	0.603	*0.076	0.247
μ*Sat60-2*	57	93	23	0.834	0.912	*0.027	0.422
Ogo1a	57	91	34	0.932	0.961	*0.020	0.522
Ogo3	49	78	28	0.928	0.934	*0.013	0.384
Ogo4	57	91	27	0.929	0.920	0.005	0.293
Average			23	0.841	0.869	*0.020	0.356
Allozymes							
mAAT-1 *	62	100	3	0.000	0.040	0.006	0.020
sAAT-3 *	61	100	2	0.406	0.486	0.031	0.131
sAAT-4 *	62	100	2	0.496	0.410	0.043	0.150
ADA-2 *	62	100	2	0.062	0.020	0.007	0.022
mH-4 *	62	100	2	0.163	0.095	0.006	0.039
sAH *	62	100	2	0.047	0.039	−0.006	0.004
ALAT *	62	100	2	0.163	0.156	−0.006	0.004
CK-A1 *	62	100	2	0.047	0.030	−0.004	0.009
CK-C1 *	56	99	2	0.000	0.030	0.005	0.015
CK-C2 *	54	99	2	0.000	0.030	0.005	0.015
FDHG *	62	100	2	0.163	0.205	−0.002	0.026
GDA *	61	100	3	0.629	0.636	−0.002	0.061
GPI-B2 *	62	100	2	0.077	0.058	−0.005	0.010
G3PDH-1 *	62	100	2	0.163	0.156	−0.007	0.004
G3PDH-2 *	61	100	3	0.141	0.160	−0.006	0.011
sIDHP-2 *	62	100	3	0.529	0.483	0.004	0.086
LDH-A1 *	61	100	2	0.124	0.242	0.022	0.074
LDH-B1 *	62	100	2	0.000	0.039	0.010	0.020
sMDH-B1,2 *	62	100	3	0.214	0.175	−0.001	0.036
MPI *	62	100	2	0.062	0.095	−0.004	0.018
PEPD-2 *	62	100	3	0.392	0.279	0.011	0.085
PEP-LT *	62	100	2	0.000	0.039	0.010	0.020
PGDH *	62	100	2	0.370	0.501	*0.098	0.228
PGM-2 *	62	100	2	0.238	0.131	0.020	0.067
Average			2.3	0.187	0.189	*0.020	0.048

genotypes not present in the baseline. These fish were called ''unknown'' and provided a more conservative estimate of the likelihood of misclassifying summer pink salmon as fall. The selection criterion was determined as the LLR at which no individuals in either test sample (baseline or unknown) were incorrectly assigned to summer or fall run.

Results

Population Genetics

Microsatellites were much more polymorphic than allozymes in the two populations. (Table 1). The number of alleles per locus ranged from 4 to 34 (mean = 23.1) for microsatellites and from 2 to 3 (mean = 2.3) for allozymes. The mean $\hat{H}_E$ for summer and fall samples was 0.87 and 0.84 for microsatellites and 0.19 and 0.19 for allozymes.

Only three allozyme loci had an $\hat{H}_E$ greater than 0.5 in one or both populations (Table 1).

Probability tests showed no significant departures from HWE in each population. When the populations were pooled, a significant P value was observed for the microsatellites ($P = 0.026$) but not for the allozymes ($P = 0.914$). Probability tests for genotypic linkage disequilibrium resulted in one significant P value ($P < 0.0001$, *Ots1* × *Ogo4*, fall run) among 42 pairwise tests of seven microsatellite loci. No significant P values were observed among 552 pairwise tests of 25 allozyme loci.

Values of $\hat{\theta}$ were similar for both marker types, and they ranged from 0.005 to 0.076 (microsatellites) and from −0.007 to 0.098 (allozymes) (Table 1). Results of the permutation tests indicated that $\hat{\theta}$ was significantly greater than zero at five mi-
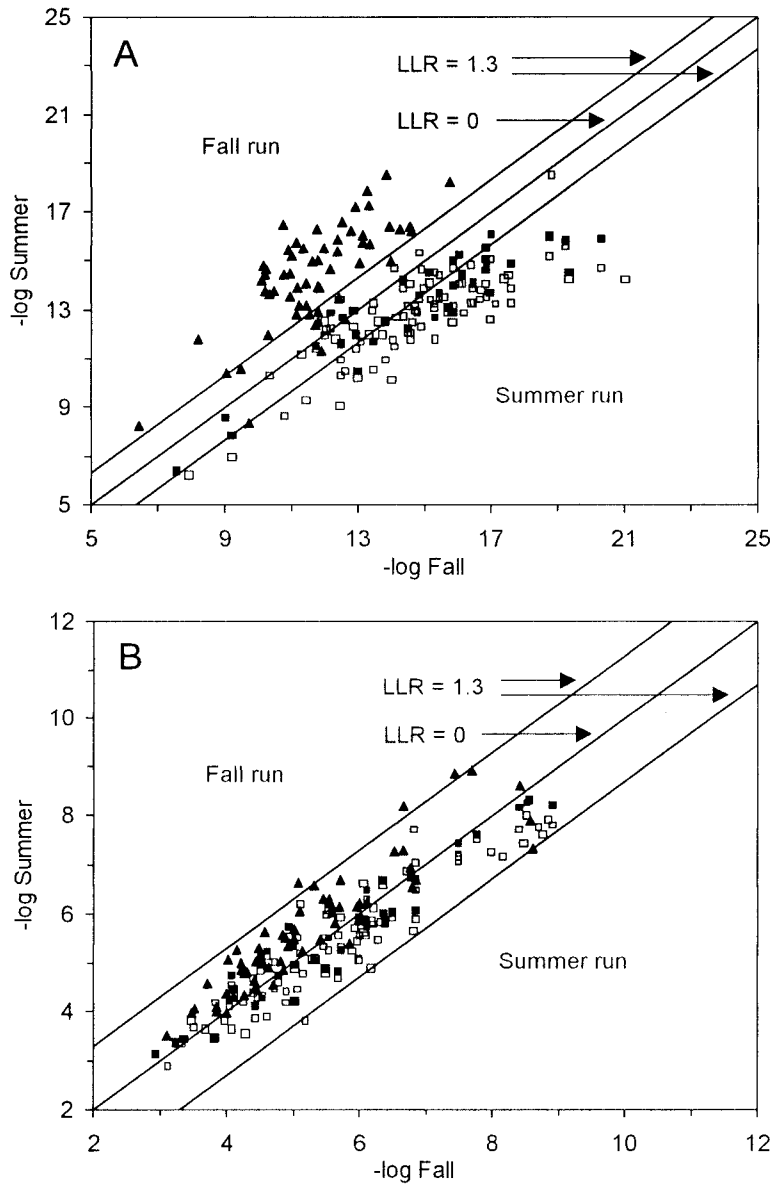
FIGURE 2.—Probability plots of multilocus genotype frequencies ($-\log_{10}$) for baseline fall run (triangles), baseline summer run (open squares), and unknown ''summer run'' (solid squares) using **(A)** microsatellites and **(B)** allozymes.

crosatellite loci and at one allozyme locus. The value of $\hat{\theta}$ over all loci was 0.020 and was significantly greater than zero ($P < 0.0001$) for both microsatellites and allozymes. Values of $\hat{f}$ and $\hat{F}$ over all loci were similar for both marker types and were not significantly greater than zero.

Population Assignment

Values of $\hat{\delta}$ varied within marker types and were higher for microsatellites (0.248–0.522) than for

allozymes (0.004–0.228) (Table 1). Probability plots of multilocus genotype frequencies showed greater delineation among populations for microsatellites than for allozymes (Figure 2). For microsatellites, the percentages of correct assignments for baseline and unknown test samples were 94.7 and 91.4% at LLR>0; 74.8 and 62.9% at LLR>1.3; 61.5 and 37.1% at LLR>2; and 31.8 and 11.4% at LLR>3. For allozymes, the percentages of correct assignments for baseline and
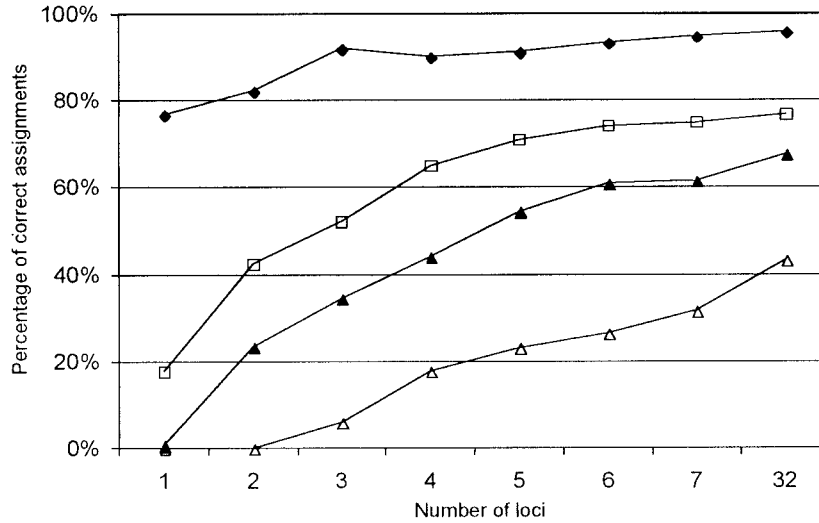
FIGURE 3.—Population assignment accuracy versus number of loci for LLR>0 (diamonds), LLR>1.3 (open squares), LLR>2 (solid triangles), and LLR>3 (open triangles). Microsatellite loci were added incrementally in order of descending $\hat{\delta}$, and all allozyme loci were added at once.

unknown test samples were 75.9 and 62.9% at LLR>0 and 3.1 and 0.0% at LLR>1.3. No correctly assigned samples exceeded a LLR of two for the allozyme loci.

The effect on population assignment accuracy produced by varying the number of loci was tested at four LLR criteria (>0, >1.3, >2, and >3) (Figure 3). Microsatellite loci were added incrementally in order of descending $\hat{\delta}$ value, and all allozyme loci were added at once. For LLR>0, the assignment accuracy, using the most informative microsatellite (*Ogo1a*), was 0.768 and was greater than the assignment accuracy using all allozyme loci. In fact, the assignment accuracy showed little increase (<3.9%) after the addition of the third microsatellite locus (Figure 3). For LLR>1.3 and >2, the increases in assignment accuracy were the greatest (24.5 and 22.5%, respectively) after addition of the second microsatellite and increased minimally (0.6 and 0.5%, respectively) after addition of the sixth microsatellite. For LLR>3, the assignment accuracy increased steadily as loci were added. Addition of the allozyme loci provided noticeable increases in individual LLR>2 and LLR>3.

Incorrectly assigned fish were those with a LLR>0 for the wrong population. Incorrect assignments occurred in 5.3% of baseline and 8.6% of the unknown test samples using microsatellites and in 24.1% of baseline and 37.1% of the unknown test samples using allozymes. In no case

did the LLR exceed 1.3 for the wrong population. Therefore, we used LLR>1.3 as our selection criterion for broodstock in 1997.

1997 Broodstock Selection

One hundred seventy-one pink salmon were genotyped using seven microsatellite loci and were assigned to one of three categories: fall run (76), indeterminate (73), and summer run (22) (Figure 4). The distribution of LLRs for individuals assigned to the fall population was 23 (1.3<LLR<2), 37 (2<LLR<3), and 16 (LLR>3). Fifty-three (69.7%) pink salmon assigned to the fall population had LLR>2.

The summer-run pinks were most prevalent in the first week of sampling and declined in number over time (Figure 4). The percentages of individuals assigned, respectively, to summer and fall populations (by week) were 23.3 and 20.9% (August 13–19), 20.5 and 44.5% (August 20–26), 5.0 and 50.5% (August 27–September 2), and 6.9 and 65.5% (September 3–17). No individuals were assigned to the summer population after September 5.

Discussion

Marker Comparison

Our comparison of marker types reveals two important practical considerations regarding their use for population assignment in salmon. First, estimators of mean allele frequency variation between populations (e.g., $\hat{\theta}$) are not reliable predictors of
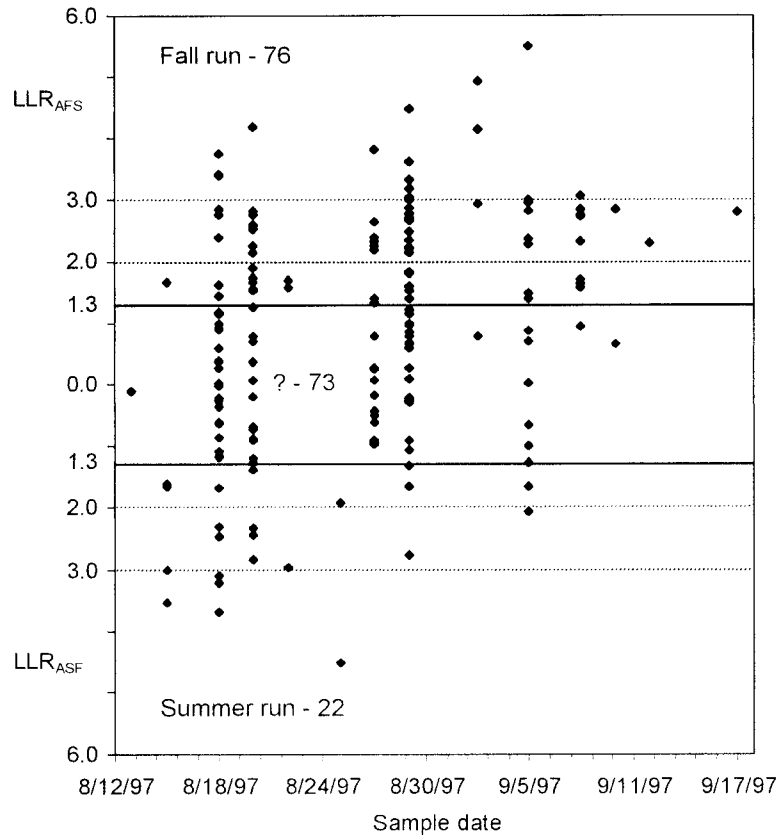
FIGURE 4.—Distribution of LLR estimates for Dungeness River pink salmon in 1997. Individuals were classified as fall run ($LLR_{AFS} > 1.3$), indeterminate ($LLR_{AFS} < 1.3$, $LLR_{ASF} < 1.3$), and summer run ($LLR_{ASF} > 1.3$), where LLR_{AFS} is the log-likelihood ratio for individual A in the fall run : summer run, and LLR_{ASF} is the log-likelihood ratio for individual A in the summer run : fall run.

our ability to determine population origin of individuals. In the present study, both marker types provide similarly low estimates of population structure ($\hat{\theta} = 0.02$). In contrast, the statistic $\hat{\delta}$, which estimates cumulative allele frequency differences and not mean variance in frequency, is larger for all microsatellite loci and is a better predictor of the assignment accuracy differences between marker types. This comparison suggests that loci with low mean variance in allele frequency can have large allele frequency differentials ($\hat{\delta}$) and be useful population affiliation markers. For populations exhibiting little genetic differentiation (such as pink salmon), loci exhibiting many alleles are preferred, because $\hat{\delta}$ is more likely to be large as a result of genetic drift at each allele (Shriver et al. 1997).

A second consideration is the number of loci to screen. Using our three most informative microsatellites ($\hat{\delta} = 0.522$, 0.422, and 0.384), we cor-

rectly assigned over 92% of baseline samples to population at LLR>0, but we found little improvement with more loci. Similarly, Estoup et al. (1998) correctly assigned over 80% (LLR>0) of individuals to population (brown trout) using the two most polymorphic loci from a seven-locus panel. We found that additional loci did not increase the proportion of individuals correctly assigned to population, but they did increase individual LLRs, thereby providing higher confidence in population assignments of some individuals. Shriver et al. (1997) selected five loci with the highest $\hat{\delta}$ (>0.65) from a panel of 1,000 loci, and, using simulation, they correctly assigned 96% of individuals to ethnic group (African American versus European American) at LLR>3. Our locus survey for Dungeness River pink salmon is not as extensive, and only one locus (microsatellite *Ogo1a*) exhibited a $\hat{\delta}$ of greater than 0.50. Had we established a LLR>3 as our selection criteria, then

only 16 individuals would have been selected for broodstock in 1997. Clearly, additional loci with high (>0.50) $\hat{\delta}$ would be needed to achieve the level of confidence (in population assignment) that is used in human forensics. Our initial results suggest that this level may be possible, but it will require researchers to survey more loci.

Although microsatellites provide greater assignment accuracy, the addition of allozymes results in a noticeable increase in individual LLR>2 and LLR>3 (Figure 3). Of the 25 allozyme loci used, 13 are detectable in skeletal muscle samples (including *PGDH**—the most informative loci), and these could therefore presumably be reliably scored in nonlethal biopsy samples. If surveying additional microsatellite loci is not possible, then adding allozymes could be considered for future broodstock screening if there is a need to increase the selection criterion.

Population Genetics and Population Assignment

Pink salmon exhibit low levels of intralineage population differentiation when compared with other Pacific salmon (Allendorf and Utter 1979). Most studies indicate that 3% or less of the total genetic variation at presumed neutral markers is a result of population differences in this species (e.g., Hard et al. 1996). Similarly, we find low (2%) but significant interpopulation genetic variation for two marker types in odd-year pink salmon from the Dungeness River. This low level of genetic differentiation is likely attributable to recent postglacial divergence and gene flow (Hard et al. 1996; Olsen et al. 1998b). Regardless, we show that reasonable estimates of population origin of individuals are possible if one uses highly variable markers and that these markers can be useful tools in achieving conservation objectives. For example, we confirmed that summer-run pink salmon were present in the lower Dungeness River after August 15. Had the selection strategy assumed that all pink salmon were fall-run salmon, the breeding program would have resulted in a number of crosses between populations. Assuming the fall- to summer-run ratio (approximately 3:1) applies to the indeterminate samples, then a random single-pair mating scheme would have resulted in approximately 56% fall × fall; 38% fall × summer; and 6% summer × summer families.

Although our results are encouraging, there are two caveats. First, the usefulness of this tool for allocating individuals to populations will likely decrease as the number of populations increases, especially if the distance between populations de-

creases (e.g., Paetkau et al. 1995; Estoup et al. 1998). Ours is a straightforward case in that aside from low genetic differentiation, there are only two populations, and they are geographically separated from other conspecific populations. Banks et al. (1996) are developing microsatellite markers that will assign individuals to four populations of chinook salmon in the Sacramento River; of these four populations, one is listed as endangered under the Endangered Species Act. In this case, it is likely that $\hat{\delta}$ will vary as a result of random drift at each marker, depending upon the population pair examined. In other words, certain loci will distinguish individuals from some, but not all, populations. In such situations, a more extensive screening of loci will probably be needed. Fortunately, microsatellites in cold-water teleosts appear to be more polymorphic than they are in mammals (Brooker et al. 1994), which suggests that suitable loci can be found for individual/population identification without the level of screening that is required for humans.

The second caveat concerns the statistical criteria for defining population identity. We used a LLR threshold (LLR>1.3) that is determined empirically from the baseline data. This approach accounts for classification error that is attributable to genotypes of similar frequency in both populations that are present in the baseline data. It does not, however, account for as yet unseen genotypes and for the sampling variance of all possible genotypes. An alternative to computing likelihood ratios is to bootstrap sample each database and to compute a 95% confidence interval for the multilocus genotype frequency in each population (Weir 1995). This method provides population assignments based on an estimate of genotypic sampling variance, but it may not improve assignment accuracy, and this should be examined in a future study.

Final Comments

In the present study, microsatellites proved useful for determining the population origin of individual pink salmon when the genetic differentiation between populations was relatively low. These results complement those of other studies that focus on more genetically divergent populations. For example, Bogdanowicz et al. (1997) showed that four microsatellites provided high resolution of continent of origin of gypsy moths from Asia and North America. They concluded that microsatellites could be useful in detecting exotic introductions of gypsy moth in North America. Paetkau et

al. (1995) showed that eight microsatellites provided high resolution of region of origin of polar bears from North America. They suggested that microsatellites could be useful for tracking migratory patterns of polar bears from these two regions. Together, the three studies highlight the value of microsatellites in assigning individuals to populations, and they demonstrate three different conservation applications for population identity data.

Finally, a comment on the supportive breeding program described in this study. The use of supportive breeding as a conservation tool has some genetic risks. These risks, such as a decrease in effective population size, are well described and warrant careful consideration (e.g., Ryman and Laikre 1991; Ryman et al. 1994). In this case, however, the resource managers determined that the risk of extinction was greater than the sum of genetic risks associated with supportive breeding. For example, the marker-based broodstock selection process may change the genetic profile of the fall-run population, because individuals with low LLR ($0 < LLR < 1.3$) are returned to the river. Further, this process may cause the two populations to become more genetically divergent. Although these are undesirable outcomes of supportive breeding, they are of less concern than the alternative of extinction of the fall-run population.

Acknowledgments

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