Original Articles

MARINE BIOTECHNOLOGY

Assignment of Sockeye Salmon (*Oncorhynchus nerka*) to Spawning Sites Using DNA Markers

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Abstract

Randomly amplified polymorphic DNA (RAPD) markers were used to assign individual adult sockeye salmon to their spawning sites using a genotype assignment test. Six primers were selected for use by screening bulked DNA samples for markers missing in fish from one or more of 5 sites in British Columbia or Alaska. Of 73 markers scored, 54 showed variation between or within sites among the sampled fish. Thirty-seven of the variable markers were not detected in any fish from one or more sites; 18 variable markers were detected in all fish from one or more other sites. Thus 25% of markers scored were found in all fish of some sites and in no fish of some other sites. An assignment test placed all 70 fish tested into their correct populations. Principal coordinate analysis of genetic variation produced clusters of fish corresponding to each sampling site. No sex-specific RAPD markers were detected among more than 1300 screened.

Key words: assignment test — sockeye salmon — RAPD — bulked DNA — principal coordinate analysis — sex-specific DNA markers

Introduction

For management purposes it is useful to be able to identify specific populations (stocks) of fish and their representation in mixed populations intercepted in test fisheries. Various allozyme and DNA markers have been used to investigate the genetic structure of salmonid populations and to assess the composition of mixed populations (reviewed by Shaklee et al., 1999; Hansen et al., 2001; Hendry et al., 2004). DNA markers can be used for rapid, accurate assessment of stock composition (Beacham et al., 2004). Assignment of individual fish to a specific population is useful for management and forensic applications, but developing discriminating markers is often challenging (reviewed by Hansen et al., 2001). In a pilot study we found that screening of bulked DNA samples is an efficient strategy to identify dominant DNA markers that are useful for assignment of fish to a particular population.

A fast and efficient method to screen for DNA polymorphisms involves randomly amplified polymorphic DNA (RAPD) markers produced by using a single short (e.g., decameric) oligonucleotide as the polymerase chain reaction (PCR) primer for both DNA strands (Williams et al., 1990; Welsh and McClelland, 1990). The amplified DNA products are classified by size as assessed by gel electrophoresis. RAPD markers variable for presence or absence are effectively dominant as their alleles are usually unknown and heterozygotes cannot be distinguished from homozygotes.

Bulking DNA samples from individuals sharing a trait is a powerful method for identification of DNA polymorphisms associated with that specific trait (Michelmore et al., 1991; Williams et al., 1993; Johnson et al., 1994; Postlethwait et al., 1999). For in-

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stance, Johnson et al. (1994) detected an average of 5 variable RAPD markers per primer by comparing bulked DNA samples of 2 laboratory strains of zebrafish (*Danio rerio*). A genetic linkage map was produced for these and other variable DNA markers (Postlethwait et al., 1994). As RAPD markers appear to be distributed throughout the genome (Williams et al., 1990; Postlethwait et al., 1994), this approach is especially useful for rapidly finding new variable markers associated with visible traits, especially for species for which little or no genetic information is available. This bulked segregant approach was used to identify and clone a polymorphic DNA marker that segregates as sex linked in rainbow trout (*Oncorhynchus mykiss*) (Iturra et al., 2001). Although DNA polymorphisms linked to the dominant male sex determination locus have been identified for several Pacific salmon species, they remain elusive for sockeye salmon (*O. nerka*) (Devlin et al., 2001).

We screened bulked DNA samples of sockeye salmon collected from 5 spawning sites having a range of geographic separation for RAPD markers missing from one fish at one or more sites. Using only 6 preselected RAPD primers, markers were identified that could be used to assign all 70 individual fish to their appropriate sampling sites. No sex-specific markers were detected in a screen of bulked DNA samples.

Materials and methods

Samples. Samples of 7 male and 7 female sockeye salmon were collected at their spawning beds at 5 locations in British Columbia and Alaska (Figure 1). To enable assessment of genetic isolation by geographic distance and to allow comparison of the ability to assign fish to sites geographically close versus distant sites, we collected fish from 2 relatively proximate sites in southern British Columbia and in northwestern Alaska, and from a site roughly equidistant between the northerly and southerly sites. The 2 most southerly sampling localities are from tributaries of the Thompson River in the lower Fraser River basin in southern British Columbia: Scotch Creek (SC) and Fennel Creek (FC). The 2 most northerly sampling localities are from the Cook Inlet in west central Alaska: Bear Creek (BC) and MacArthur River (MR) on the south and north sides of the inlet, respectively. The fifth sampling locality is approximately equidistant from the southerly and northerly sampling sites in the Stikine River system of northern British Columbia: Tahltan Lake (TL). Samples were collected from British Columbia in August and September 1995 and from Bear Creek in 1992. The date of collection from MacArthur River is unknown. Samples of liver (or muscle for MR) were stored frozen prior to and after being transported to Simon Fraser University for analysis.

DNA purification. Template DNA from the 3 spawning locations in British Columbia was isolated from frozen liver samples by phenol-chloroform extraction after digestion with proteinase K (Corley-Smith et al., 1996). DNA from the Alaskan samples was purified using the QIAamp Tissue Kit (Qiagen). Precipitated DNA was resuspended in TE buffer (10 mM Tris-HCl, 0.1 mM ethylenediaminetetraacetic acid, pH 8.0). DNA was quantified by spectrofluorometry with Pico-Green (Molecular Probes), using *Hind*III fragments of λ DNA (Gibco BRL, 15612-013) as standards. DNA samples were analyzed by electrophoresis on agarose gels to ensure that they were largely intact and were tested for RAPD amplification using one or more primers that produced some monomorphic markers shared by all tested samples. Equal-sized aliquots of DNA at 0.5 ng/μl from 7 fish of each sex from each site were bulked (pooled together) and used as template for bulked DNA screening.

RAPD DNA amplification. DNA amplification via PCR was performed in an Idaho 1605 Air Thermocycler (Idaho Technology), as described by Corley-Smith et al. (1997). The program commenced with 2 cycles of 60 seconds at 91°C, 7 seconds at 42°C, and 70 seconds at 72°C, followed by 38 cycles of 1 second at 91°C, 7 seconds at 42°C, and 70 seconds at 72°C and was terminated with a 4-minute hold at 72°C. Ramp times for heating and cooling were each 9°C per second. Reactions were carried out in heat-sealed glass capillary tubes with a 10-μl volume containing 1× Idaho 3 mM Mg buffer, 100 μM of each dNTP (Pharmacia), 0.06 U/μl of *Taq* DNA polymerase (Promega, in storage buffer B), 0.8 μM of a decameric RAPD oligonucleotide primer, and 4 ng of template DNA. For every set of PCR reactions, negative controls were included to test for self-amplification and DNA contamination. Each primer was also tested in RAPD reactions using a 10-fold range of DNA concentrations on a large subset of samples; the patterns were the same. The Nucleic Acid Protein Service (NAPS) unit at the University of British Columbia produced the sets of random decameric oligonucleotide RAPD primers (B.C.200–400). For the data reported here the following primers were used: 301 (CGGTGGCGA), 305 (GCTGGTACCC), 307 (CGCATTTGCA), 308 (AGCGGCTAGG), 314 (ACTTCCTCCA), and 315 (GGTCTCCTAG).

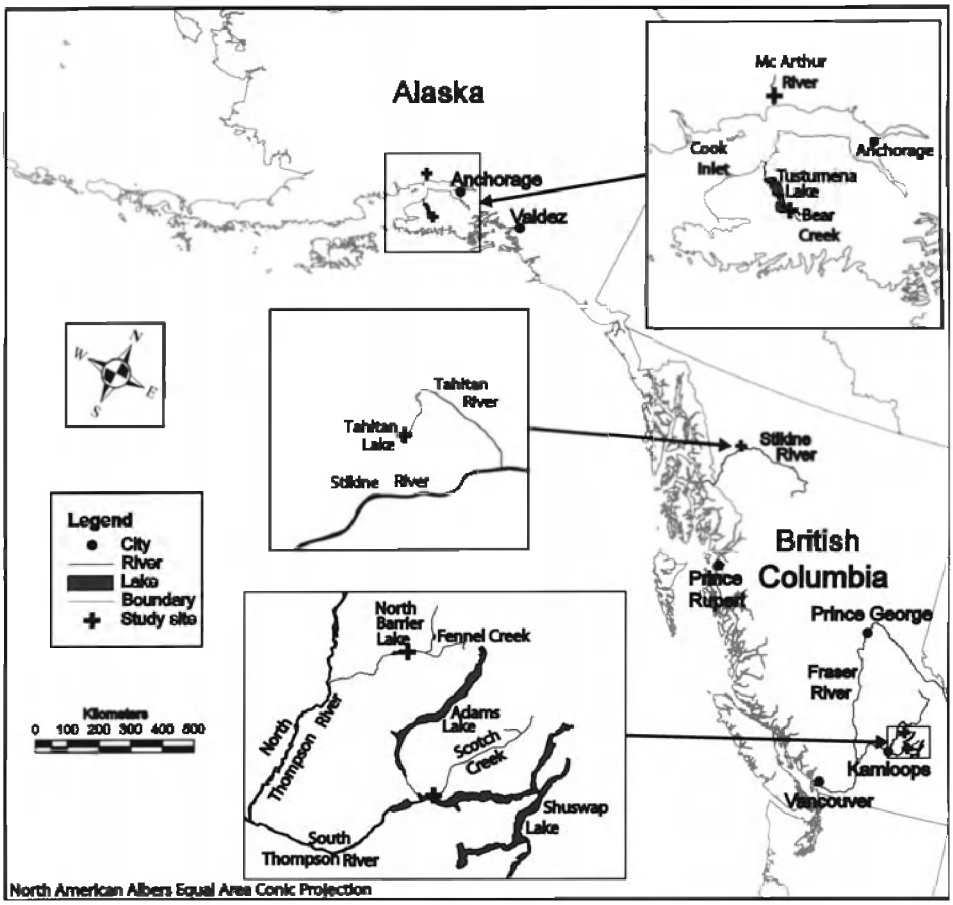

Fig. 1. Locations of spawning sites for sockeye salmon used: Scotch Creek (SC), South Thompson River water shed; Fennel Creek (FC), North Thompson River water shed; Tahltan Lake (TL), Stikine River system; and MacArthur River (MR) and Bear Creek (BC) on north and south sides of Cook Inlet, respectively.

Analysis of RAPD-PCR products. The PCR products were separated by electrophoresis on agarose gels. Ten microliters of each PCR reaction was loaded onto 1.8% agarose gels in 0.5 × TBE (0.045 M Tris, 0.045 M boric acid, 0.001 M EDTA) containing 0.5 μg/ml ethidium bromide; 100-bp DNA ladders (BRL) were loaded into multiple lanes as size standards. Electrophoresis was performed at 3 V/cm. Ethidium bromide staining was visualized with a 302 nm transilluminator. The digital images were captured on a UVP Gel Documentation System, and sizes (in base pairs) of each amplified fragment were assigned using the NCSA GelReader program, Macintosh Version 2.0.5 (National Center for Supercomputing Applications, University of Illinois at Urbana-Champaign). RAPD markers were scored as either present or absent; in one instance (noted in Table 1) presence or absence was not certain. All RAPD amplifications and product analyses were performed at least twice on different occasions, with no disagreement except as noted. Two readers independently scored the RAPD products for size and presence or absence (or ambiguity). Some amplicons showed possible ambiguities in interpretation of presence or absence; they were excluded from the analysis. In addition to size markers, to facilitate comparison and identification of bands, each gel included one or more lanes corresponding to the repeat of a previously performed RAPD reaction. In this way about 10% of the samples were resampled; no genotyping errors were detected.

Markers were named according to the convention adopted for the zebrafish RAPD linkage map (Postlethwait et al., 1994): that is, by the RAPD primer used and the estimated size. For example, 301B.C.1461 is a marker amplified with primer 301 from the University of British Columbia RAPD primer set, and 1461 is its estimated size (in base pairs). Markers were assessed for presence or absence in bulked or individual DNA samples. In addition, RAPD markers restricted to male or female fish were sought. Sex was determined by dissection and observation of male or female gonads.

Data analysis. An assignment test (Paetkau et al., 1995, 1997; Waser and Strobeck, 1998) was applied to the marker data, using the Assignment Calculator (Doh) found at http://www.biology.ualberta.ca/jbrzusto/Doh.php. The test assumes that the genetic loci are independent and that each

Table 1. Frequencies of 54 RAPD markers showing variation in sockeye salmon from five sampling localities in British Columbia and Alaska

	Sampling site[a]				
Marker	*MR*	*BC*	*TL*	*FC*	*SC*
301B.C.1461	1.0	0.6	0.1	1.0	1.0
301B.C.1349	1.0	0.6	0.1	1.0	1.0
301B.C.1225	1.0	0.6	0.0	1.0	0.9
301B.C.1142	1.0	0.9	1.0	1.0	1.0
301B.C.1013	0.0	0.1	0.0	0.1	0.1
301B.C.754	0.2	0.7	1.0	1.0	1.0
301B.C.566	1.0	0.7	0.1	1.0	1.0
305B.C.823	0.9	0.6	0.6	0.9	0.9
305B.C.531	0.0	0.9	0.9	0.0	0.0
307B.C.918	0.3	0.1	0.0	0.4	0.0
307B.C.894	0.1	0.0	0.9	0.0	0.0
307B.C.878	0.0	1.0	0.0	1.0	0.9
307B.C.855	1.0	0.8	0.5	0.0	0.0
307B.C.844	0.0	0.0	0.7	0.0	0.6
307B.C.824	0.0	1.0	0.0	0.0	1.0
307B.C.811	0.8	0.0	0.9	0.4	0.0
307B.C.796	0.0	0.1	0.0	0.0	0.0
307B.C.782	0.0	0.0	0.8	0.0	0.3
307B.C.768	0.0	0.8	0.0	0.0	0.0
307B.C.734	1.0	0.0	0.0	1.0	0.0
307B.C.708	0.0	1.0	1.0	0.0	1.0
307B.C.581	0.0	0.8	1.0	0.0	0.0
308B.C.876	1.0	0.8	1.0	1.0	1.0
308B.C.817	0.0	0.6	0.8	0.0	0.4
308B.C.781	0.2	0.5	0.1	0.0	0.9
308B.C.761	1.0	0.4	0.6	1.0	0.0
308B.C.742	0.0	0.0	0.3	0.0	0.4
308B.C.687	0.0	1.0	0.0	1.0	1.0
308B.C.668	1.0	0.9	1.0	0.0	0.0
308B.C.641	1.0	0.0	1.0	0.0	1.0
308B.C.613	0.0	0.6	0.0	0.0	0.0
308B.C.598	1.0	0.6	1.0	1.0	1.0
308B.C.545	1.0	0.9	1.0	0.0	1.0
308B.C.515	0.0	1.0	0.0	0.0	1.0
308B.C.503	1.0	1.0	1.0	1.0	0.0
308B.C.470	0.0	1.0	1.0	1.0	1.0
308B.C.455	1.0	0.0	1.0	0.0	0.0
308B.C.411	0.0	1.0	0.0	0.0	0.0
308B.C.387	1.0	1.0	1.0	0.0	0.0
308B.C.296	0.0	0.6	0.0	0.0	0.0
314B.C.1295	0.0	0.0	0.0	0.6	0.8
314B.C.937	0.9	0.5	0.8	1.0	0.6
314B.C.893	1.0	1.0	1.0	0.6	0.6
314B.C.842	0.9	0.5	0.9	0.9	0.9
314B.C.745	0.0	0.0	0.8	0.4	0.8
314B.C.587	1.0	0.8	1.0	0.8	1.0
314B.C.535	0.0	0.0	0.1	0.0	0.0
314B.C.506	0.6	0.0	0.0	0.0	0.1
314B.C.481	1.0	1.0	1.0	—[b]	0.9
315B.C.1286	0.3	0.6	1.0	1.0	0.8
315B.C.851	0.0	0.0	0.1	0.0	0.0
315B.C.702	1.0	0.9	1.0	1.0	1.0
315B.C.317	0.1	0.2	0.1	0.6	0.3
315B.C.262	0.7	0.9	0.5	0.4	0.6

[a]Site names: MacArthur River (MR), Bear Creek (BC), Tahltan Lake (TL), Fennel Creek (FC), and Scotch Creek (SC).
[b]Indicates incomplete data.

Table 2. Assignment of individual fish to sampling sites

Site	*Assn. Prob.*	*MR*	*BC*	*TL*	*FC*	*SC*
MR	0.0018–0.130	0.0	110	3900	600	6400
BC	0.0010–0.011	9.4	0.0	3900	6300	6300
TL	0.0075–0.130	7.6	7.5	0.0	3200	3200
FC	0.0028–0.120	7.0	8.2	10.7	0.0	370
SC	0.0006–0.044	10.4	6.6	9.4	6.2	0.0

[a]The first column shows the range of assignment probabilities for individual fish to their home population (collection site). The logs of interpopulation genetic distances are shown below the diagonal for each pairwise combination of populations. Approximate distances (km) by water between sampling sites are shown above the diagonal.

population has Hardy-Weinberg frequencies of genotypes. The calculator uses the genotypes of individuals from each sample set (spawning site) to calculate a probability of assignment of each individual to each sample set and assigns it to the most probable set after removing the effect of the assigned individual's genotype on its sample set. It also calculates pairwise interpopulation distances, a measure of the dissimilarity of the gene pools of the sample sets being compared. The calculator was run with 1000 randomizations, allele frequencies were adjusted to avoid zeros according to Titterington et al. (1981), and the ploidy was set as 1 because the markers are treated as dominant. The Mantel test (Manly, 1993; Sokal and Rohlf, 1995) was used to test for correlation of the interpopulation genetic distances with geographic distances. The test was performed with PopTools, Version 2.5, build 5 (http://www.cse.csiro.au/poptools/). Principal coordinate (PCO) analysis was performed with MVSP Version 3.0 (Kovach Computing Services) using Gower's general similarity coefficient. Individuals and marker (314B.C.481) for which data were missing were excluded.

Results

Bulked DNA samples were initially prescreened with RAPD primers to identify primers that produced markers that appeared to be polytypic between sampling locations. For each population, 2 samples (one for each sex, including DNA pooled from 7 fish) were tested. From 15 primers tested, 6 were selected that consistently produced well-defined bands of which at least one appeared to be missing from the bulked DNA samples from one or more sites. These 6 primers were then applied to individual DNA samples from each of 14 fish from each spawning site. Of 73 RAPD bands scored, 54 were variable among the sampled fish (Table 1). Of these, 37 were absent in all fish tested from at least one site, confirming the sensitivity of the screen of bulked DNA, while 18 were present in all 14 sampled fish of at least one site. Thus 25% of markers scored were found in all fish of some sites, and in no tested fish of at least one site. There were 7 markers restricted to a single site, but 3 of these were detected in only 1 or 2 fish from that site. One marker (308B.C.411) was observed in all fish tested from Bear Creek but in no fish from other sites, indicating it may be a population-specific marker that is fixed, though a larger sample size is required to confirm that possibility.

The Assignment Calculator assigned all individual fish to their corresponding spawning sites with no exceptions; the ranges of probabilities of assignment to the home site are shown in Table 2. The probability of assignment of any fish to another site was less than 1/1000th of the probability of assignment to its home site. The interpopulation distances between each sampling site estimated by the Assignment Calculator are shown in Table 2, along with geographic distances. The Mantel test indicated that genetic and geographic distance are not significantly correlated ($r = -0.026$, $P = 0.51$) for the samples from the 5 tested sites.

PCO analysis of the genetic differences was performed on the data set (Figure 2). The first two

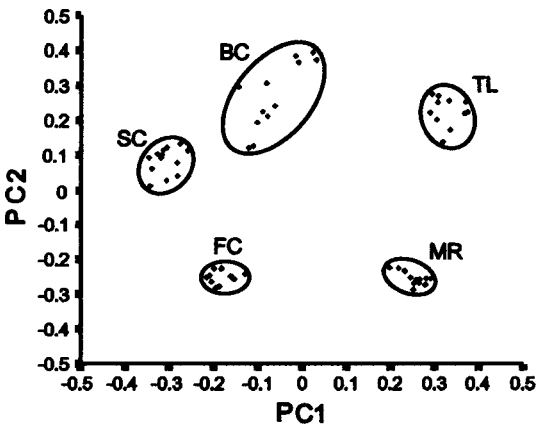

Fig. 2. Principal coordinate analysis of genetic differences between sampled populations. The first two principal coordinates are shown as the PC1 and PC2 axes. Samples from each spawning site formed clusters and are enclosed within an ellipse. Site names: MR, MacArthur River; BC, Bear Creek; TL, Tahltan Lake; FC, Fennel Creek; and SC, Scotch Creek.

principal coordinates account for only about 40% of the genetic variation, but the samples from each of the spawning sites cluster together and are clearly distinguished. The first principal coordinate (PC1) distinguishes the southerly Thompson River populations (SC and FC) from 2 of the northerly populations (TL and MR), but not from the Bear Creek population. Inclusion of PC2 clearly distinguishes the SC from the FC populations, as well as TL from MR, and BC from MR (Figure 2). Geographically proximate populations were not clustered near each other on the PCO plot, consistent with a lack of correlation of genetic and geographic distances for these samples.

No sex-specific RAPD marker was detected among more than 300 markers analyzed in individual fish from the 5 sampling sites, or in the analysis of bulked DNA samples. In addition, we screened over 1000 RAPD markers with more than 100 primers using bulked DNA samples from 7 males and 7 females from each of the 3 sites in British Columbia, but detected no sex-specific markers.

Discussion

The successful assignment of all individual fish to their spawning sites, and the interpopulation distances calculated by the assignment test, indicate that there is considerable genetic differentiation among the 5 sampling localities of sockeye salmon. RAPD screening of bulked DNA samples appears to be an efficient and successful method to find markers useful for assignment of individual fish to their site of origin. Only 15 primers applied to bulked DNA samples from the 5 collection sites were required to identify the 6 primers used in this investigation to assign individual fish to their sites.

Our data show genetic differentiation of proximate sockeye populations within the Thompson River system and on opposite sides of Cook Inlet. There is much evidence for limited gene flow among sockeye populations even on a small geographic scale (reviewed by Hendry et al., 2004). Studies of allele frequencies indicate that there can be reproductive isolation among sockeye spawning at discrete sites within the same nursery lake system, specifically Iliamna Lake (Varnavskaya et al., 1994) and Lake Washington (Hendry et al., 1996, 2000). Microsatellite allele frequencies suggested reproductive isolation of 3 populations of sockeye separated by less than 50 km in Barkley Sound (Nelson et al., 1998).

Genetic differentiation of salmon populations depends on homing. Substantial straying would undermine this population structure unless the strays have low reproductive success. Sockeye salmon home to natal streams and incubation sites with considerable fidelity; straying can occur, but it is difficult to measure (Quinn et al., 1987; Griffith et al., 1999; Hendry et al., 2004). Analyses of the microstructures of otoliths indicated straying of only 0.1% to 1% for sockeye populations incubated at discrete sites in Lake Washington (Quinn et al., 1999). Homing restricts gene flow and can cause sufficient reproductive isolation to allow for genetic differentiation of populations via selection for specific adaptive traits (Hendry et al., 2004). Introduced sockeye can rapidly evolve reproductive isolation and adaptively diverge (Hendry et al., 2000; Hendry, 2001). Genetic differentiation can arise and be maintained if the particular markers being surveyed are under selection or are physically linked to loci under selection (Hendry, 2004). Such markers are valuable for our purpose of assigning fish to particular populations, but are probably not neutral markers, and thus are inappropriate to use for estimations of gene flow. Founder effects and genetic drift can also contribute to the population structure of sockeye salmon (Adkison, 1995; Ramstad et al., 2004).

Microsatellite DNA markers are useful for assessing population structure, genetic differentiation, stock composition, and gene flow. The number of useful microsatellite loci is increasing for salmonids, but their identification remains labor-intensive. Other types of DNA markers provide the opportunity to sample larger numbers of loci; these include RAPDs, amplified fragment-length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs). In a methodologic comparison, microsatellite, randomly selected RAPD, and most allozyme markers produced concordant estimates of genetic variation within and between 4 stocks of sockeye salmon collected in Cook Inlet, though few RAPD markers were assessed (Allendorf and Seeb, 2000). RAPD markers are especially useful when applied to species for which there is little molecular genetic data, and they can be used to quickly screen for new variable markers. AFLP markers offer advantages similar to RAPDs and have been reported to be highly reproducible (Jones et al., 1997; Vos et al., 1995). A study on whitefish (*Coregonus clupeaformis*) indicated that AFLP markers were much more efficient than microsatellite markers for assigning individuals to populations (Campbell et al., 2003). In a model of 2 populations, the probability of correct assignment increased as the number of loci increased and as the differential in frequencies of the markers between the populations increased, especially as the frequencies approached 0% or 100%

for one of the populations (Campbell et al., 2003). Similar arguments for RAPD markers should apply, and screening of bulked DNA permits the efficient identification of discriminating RAPD or AFLP markers. The ability to assign an individual to a population, or exclude it from other defined populations, within confidence limits, is a useful tool for forensic investigations (Waser and Strobeck, 1998; Cornuet et al., 1999).

Several concerns about data interpretation arise when using RAPD markers. As dominant markers, departure from Hardy-Weinberg equilibrium is impossible to evaluate. There have been reports of inconsistent detection of RAPD markers for identical samples at different laboratories. This problem can be minimized by adherence to standardized conditions and by the use of a sufficient excess of primer and good quality template DNA (discussed by Corley-Smith et al., 1997). For the present study all DNA samples used as templates in PCR were first assessed by electrophoresis to ensure intactness and were pretested by PCR amplification with primers that produce several strong monomorphic products. The monomorphic markers served as positive controls for PCR and template quality. The thermocycler we used provides highly uniform temperature conditions that may not be obtained with some cyclers. There have been concerns about the identification of RAPD bands as homologues based on identical electrophoretic mobility on agarose gels. This concern was alleviated by comparison of bands with closely spaced size markers on multiple lanes, monomorphic markers on the same lane, and the products of other RAPD reactions on other lanes of the same gel. The possible overlap of nonhomologous RAPD bands would result in uncertainty in assigning a marker to a specific locus (Karp et al., 1997). This uncertainty can be resolved by pedigree analysis (Clark and Lanigan, 1993; Johnson et al., 1994, 1996; Corley-Smith et al., 1996, 1997), but that was not feasible in this study. The anticipated and observed number of amplicons for each primer is limited, making overlap unlikely. Pedigree analysis of zebrafish RAPD markers indicated that most are unique, dominant markers for which corresponding alleles are not detected, but about 10% of RAPD alleles are co-dominant (Johnson et al., 1994, 1996; Corley-Smith et al., 1996).

RAPD products can be resolved to single nucleotide discrimination on polyacrylamide sequencing gels, and the use of fluorescent primers can increase the sensitivity of detection (Corley-Smith et al., 1997). This also allows the products of multiple PCR reactions to be compared with internal size standards on a single lane or capillary of an automated sequencer, increasing throughput. Useful RAPD amplicons can be cloned and sequenced to design locus-specific primers for the detection of DNA polymorphisms under stringent conditions, eliminating the possibility of misidentification by overlap of nonhomologous replicons (Paran and Michelmore, 1993; Postlethwait et al., 1999; Iturra et al., 2001).

The 100% assignment success in this pilot study was achieved by using data from 6 prescreened primers. Successful assignment could have been achieved with fewer primers, but we did not want to overextend a conclusion based on a small sample size. Using the same samples for assignment and selection of primers may have slightly biased the assignment success upward. Nevertheless, the 100% assignment success with low probability of misassignment observed in this pilot study based on a few selected primers indicates the method warrants further assessment.

We surveyed more than 300 RAPD markers in individual fish without detecting any markers that were sex-specific. In addition, over 1000 markers were assessed using bulked male and female sockeye DNA from 3 sites, and none appeared to be sex-specific. To test whether RAPD analysis of bulked samples provides a sufficiently random and efficient method to detect sex-linked markers, it was applied to mouse DNA. The fraction of markers found and confirmed to segregate as male-specific corresponded to the 5% of the mouse genome thought to be in the nonpseudoautosomal and nonrecombining region of the Y chromosome. Subsequent testing confirmed these markers to be male-specific in another mouse strain. As we screened over 1300 markers in bulked male and bulked female sockeye DNA, our coverage was approximately 46 markers per chromosome. Assuming an even distribution of RAPD markers, the fraction of the sockeye genome restricted to either sex is less than 0.1% (1 searched-for locus in 1300 markers is 0.08%), much less than in mice.

In conclusion, screening bulked DNA for polytypic markers is an effective method to efficiently identify genetic markers useful for assignment of sockeye salmon to their populations. This approach should be particularly useful when applied to species for which there is less population differentiation than for philopatric salmonids.

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