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PRIMER NOTE

Usefulness of heterologous microsatellites obtained from brook charr, *Salvelinus fontinalis* Mitchill, in other *Salvelinus* species

B. ANGERS*† and L. BERNATCHEZ†

**Département des sciences de l'environnement, Université du Québec à Montréal, C.P.8888, Succursale Centre-Ville, Montréal Canada H3C 3P8 and †Département de biologie, Université Laval, Sainte-Foy Canada G1K 7P4*

Microsatellite loci are increasingly showing great potential utility for numerous applications in evolutionary and conservation genetics (Bruford & Wayne 1993; Queller *et al*. 1993; Paetkau & Strobeck 1994; Taylor *et al*. 1994). A major constraint in the use of microsatellites is the need to isolate and characterize them from cloning and sequencing techniques when it has not been done for the species of interest. One way to circumvent this step is to screen variation in microsatellites developed for other species in order to find potential useful primers. However, usefulness of heterologous primers is generally uncertain, except that it has been shown that polymorphism at a given locus may decrease with increasing phylogenetic distance from the source species (Moore *et al.* 1991; FitzSimmons *et al*. 1995). Recently, we have reported the characterization of microsatellite loci developed for brook charr *Salvelinus fontinalis* Mitchill (Angers *et al.* 1995), a member of the salmonid family. The *Salvelinus* genera comprises five other recognized species, most of them being economically important and showing a wide range of geographical distribution. As no microsatellite loci are actually published for other *Salvelinus* species, the demonstration of usefulness of heterologous primer pairs of microsatellite loci designed for brook charr would be valuable in providing additional genetic markers for these species.

Dinucleotide microsatellite loci were previously isolated from a partial genomic library of brook charr (Angers *et al.* 1995). Eight primer pairs were tested on four individuals (except for Juneau Lake; $n = 3$) from two geographically distant populations in each species (Table 2). Screening of variation and PCR conditions were performed as described in Angers *et al.* (1995) for all loci. Primer sequences for SFO-11 which was not described before are 5′-GGTTTCATGCATTTGTCTGT and 5′-CTCGAAAAAATCACCAATTAC. PCR conditions are the same as for other loci and primers annealing temperature is 60 °C.

Polymorphism was measured as the number of alleles per locus (A) and expected heterozygosity (H_E). Analysis of heterogeneity in allelic frequencies (Sokal & Rohlf 1981) between populations within species was assessed using the MONTE program of the REAP software package (McElroy *et al.* 1992) which performed chi-square randomization tests (Roff & Bentzen 1989) for all pairwise comparison of populations at all individual loci with 1000 randomizations. Probability of identity within species was calculated using observed allelic frequencies, according to Paetkau & Strobeck (1994).

For each *Salvelinus* species, all primer pairs amplified a PCR product corresponding to an homologous microsatellite locus, with the same PCR conditions used for brook charr. Levels of polymorphism was highly variable among loci and species. Loci SFO-1, SFO-3 and SFO-20 with long repeat arrays (65, 50 and 60 repeats, respectively) remained monomorphic across all species with an allelic size of 184, 248 and 172 bp. Locus SFO-11 with 10 (GT) repeats in *S. fontinalis*, was also monomorphic within each species, but with different alleles among species. Two loci, SFO-12 and SFO-18, revealed moderate levels of variation, except for *S. alpinus* and *S. confluentus* that were monomorphic at SFO-12, and *S. malma* at SFO-18 (Table 1). Locus SFO-18 was exceptionally highly polymorphic in *S. leucomeanis* with 12 alleles for eight individuals ($H_E = 0.90$). SFO-8 and SFO-23 were highly variable in all *Salvelinus* species, with 6–10 alleles by species and expected heterozygosity ranging from 0.71–0.89 (Table 1).

Some loci revealed clear differentiation in allelic sizes among species. Thus, SFO-11 was fixed for allele 126 for *S. fontinalis*, 124 for *S. namaycush* and 122 for other charrs (Table 2). Allelic size distribution of SFO-12 and SFO-18 showed little or no overlap among species. Overlap in allele size was observed at SFO-12 and SFO-18 between *S. fontinalis* and *S. namaycush*, but not with other charrs. *S. confluentus* was fixed for allele 229 at locus SFO-12, *S. alpinus* for allele 225 while alleles 223 and 225 are found in *S. malma*. *S. malma* was fixed for allele 155 at locus SFO-18, while alleles 161 and 163 were found in *S. alpinus* and alle-

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Correspondence: Louis Bernatchez, Département de biologie, Université Laval, Sainte-Foy, Canada G1K 7P4, Canada. Fax: (418) 656 2043 E-mail: Louis. Bernatchez@bio.ulaval.ca

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Table 1 Sample size (*n*), number of alleles (A), expected heterozygosity (H_E) and probability of identity at five locus in *Salvelinus* species. Results for *S. fontinalis* are from Angers *et al.* (1995) except for locus SFO–11

		SFO-8		SFO-11		SFO-12		SFO-18		SFO-23		
Species	*n*	*A*	H_E	*A*	H_E	*A*	H_E	*A*	H_E	*A*	H_E	Probability of identity
S. fontinalis	100	18	0.87	1	0.00	6	0.53	5	0.58	16	0.86	1/13 572
S. namaycush	8	7	0.79	1	0.00	2	0.38	5	0.63	10	0.86	1/4859
S. malma	7	7	0.83	1	0.00	2	0.46	1	0.00	10	0.89	1/4344
S. alpinus	8	9	0.83	1	0.00	1	0.00	2	0.38	8	0.71	1/437
S. confluentus	8	9	0.86	1	0.00	1	0.00	2	0.38	6	0.77	1/736
S. leucomaenis	8	8	0.83	1	0.00	3	0.48	12	0.90	10	0.87	1/166 663

Table 2 Sample size (*n*), number of alleles (*A*) per locus and range of allele size range for each population. Results for *S. fontinalis* are from Angers *et al.* (1995) except for locus SFO-11. $^*P \leq 0.1$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ for the analyses of heterogeneity in allelic frequencies. GenBank accessions numbers for primer sequences are: U50302–U50306

		SFO-8		SFO-11		SFO-12		SFO-18		SFO-23	
Species/Populations	*n*	*A*	Size (bp)	*A*	Size (bp)	*A*	Size (bp)	*A*	Size (bp)	*A*	Size (bp)
S. fontinalis (Brook charr)											
Mauricie (Québec)	100	18	204–276	1	126	5	249–275	5	175–185	16	148–190
S. namaycush (Lake trout)											
Haliburton L. (Ontario)	4	3***	276–290	1	124	2***	259–261	3	173–187	6***	192–234
Marquette (Michigan)	4	4	268–284	1	124	1	261	4	173–193	5	206–250
S. malma (Dolly varden)											
Juneau L. (Alaska)	3	5	262–304	1	122	2***	223–225	1	155	4***	196–248
Kamchatka R. (Russia)	4	4	262–280	1	122	1	223	1	155	6	254–276
S. alpinus (Arctic charr)											
Français L. (Québec)	4	5	278–290	1	122	1	225	1***	161	1***	218
Thun L. (Switzerland)	4	6	252–284	1	122	1	225	2	161–163	7	136–186
S. confluentus (Bull trout)											
UpperArrow L (B.C.)	4	4***	234–244	1	122	1	229	2***	151–157	4***	224–236
Metiolus R. (Oregon)	4	5	268–296	1	122	1	229	1	157	3	226–248
S. leucomaenis (Japanese charr)											
Belaya R. (Russia)	4	5*	262–288	1	122	2**	225–235	5***	209–289	6**	140–222
Sokol'nikovka (Japan)	4	5	250–278	1	122	2	203–225	7	199–283	5	152–236

les 151 and 157 in *S. confluentus*. No overlap was observed at SFO-18 between *S. leucomaenis* and other species (Table 2).

Significant population differentiation was observed in all species. Thus, comparisons of allele frequency distribution at polymorphic loci revealed significant heterogeneity in 13 of the 17 pairwise comparison ($P < 0.01$) (Table 2). In some cases, there was no shared alleles among populations (e.g. *S. confluentus* at SFO-8, *S. alpinus* and *S. malma* at SFO-23, *S. leucomaenis* at SFO-18). High probability of individual identity was observed in all species. Thus, all individuals analysed had unique composite genotype. The probability that two individuals drawn at random from a given group shared the same genotype ranged from 1/437 to 1/166 663 (Table 1).

Given the small sample sizes used in this study, the objective was not to generate precise estimates of genetic diversity of microsatellites in *Salvelinus* species, but instead to provide a relative quantification of the potential usefulness of different loci to address genetic issues in this genera. This approach revealed that five of the eight loci isolated from brook charr can potentially be used to address different genetic questions in *Salvelinus*. Thus, microsatellite SFO-11 revealed fixed differences among some species and consequently could be used as a specific diagnostic locus. Although not fixed, loci SFO-12 and SFO-18 could be useful for the same purpose for species where allelic size classes were not overlapping. Loci SFO-8, SFO-12, SFO-18 and SFO-23 generally showed moderate to high genetic diversity which had a significant interpopulation variance component. This indicated their utility for intra- and interpopulation diversity analyses. Finally, high

probability of identity indicated that these microsatellite loci have potential for identifying individuals and makes them useful in a variety of applications including analysis of paternity, family relatedness, and gene mapping. In summary, these microsatellites provide additional markers to address a broad range of genetic issues in all *Salvelinus* species, from individuality to interspecific interactions.

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