Molecular Ecology Notes (2005) **5**, 346–351 doi: 10.1111/j.1471-8286.2005.00922.x

PRIMER NOTE

Isolation and cross-familial amplification of 41 microsatellites for the brook charr (*Salvelinus fontinalis*)

GUY M. L. PERRY,* TIM L. KING,† JÉRÔME ST.-CYR,* MANON VALCOURT* and LOUIS BERNATCHEZ*

**Département de biologie, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4, †United States Geological Survey–Biological Research Division (USGS-BRD), Leetown Science Center, Aquatic Ecology Branch, 11649 Leetown Road, Kearneysville, WV, USA 25430*

Abstract

The brook charr (*Salvelinus fontinalis*; Osteichthyes: Salmonidae) is a phenotypically diverse fish species inhabiting much of North America. But relatively few genetic diagnostic resources are available for this fish species. We isolated 41 microsatellites from *S. fontinalis* polymorphic in one or more species of salmonid fish. Thirty-seven were polymorphic in brook charr, 15 in the congener Arctic charr (*Salvelinus alpinus*) and 14 in the lake charr (*Salvelinus namaycush*). Polymorphism was also relatively high in *Oncorhynchus*, where 21 loci were polymorphic in rainbow trout (*Oncorhynchus mykiss*) and 16 in cutthroat trout (*Oncorhynchus clarkii*) but only seven and four microsatellite loci were polymorphic in the more distantly related lake whitefish (*Coregonus clupeaformis*) and Atlantic salmon (*Salmo salar*), respectively. One duplicated locus (*Sfo228Lav*) was polymorphic at both duplicates in *S. fontinalis*.

Keywords: brook charr, *Clupeaformis*, microsatellites, *Oncorhynchus*, *Salmo coregonus*, *Salvelinus fontinalis*

Received 10 November 2004; revision accepted 7 January 2005

The brook charr, *Salvelinus fontinalis*, is found throughout the Northern Hemisphere including North America, Continental Europe and the British Isles, its distribution being determined by historical glaciation and subsequent re-invasion (Danzmann *et al*. 1998; Brunner *et al*. 2000). As a whole, brook charr and other members of the genus *Salvelinus* show considerable lability in the exploitation of habitats or life history including differentiation along several alternate adaptive phenotypic axes dealing with anadromy, depth selection, and feeding type (Gislason *et al*. 1999; Jonsson & Jonsson 2001). Molecular variation tends to reflect the above adaptive phenotypic variation either on gross geographical scales (i.e. Castric *et al*. 2001) or by more specific environmental niche characteristics (Gislason *et al*. 1999; Castric *et al*. 2001), suggesting a tendency for rapid population subdivision and development of genetic substructure. A number of microsatellites have been developed for the brook charr (Angers *et al*. 1995; Angers & Bernatchez 1996) but the relative dearth of molecular tools for the documentation of genetic variation in *S. fontinalis* compared to other salmonids forces much molecular work in this genus to rely on cross-amplification of microsatellites from other salmonid species. We present sequence information, amplification success and polymorphism for 41 brook charr microsatellites including data on cross-amplification in other salmonid species.

Correspondence: Guy M. L. Perry, Fax: 1-418-656-7176; E-mail: guy.perry@giroq.ulaval.ca

Genomic DNA from *S. fontinalis* was phenol–chloroform-extracted (Sambrook *et al*. 1989) from approximately 50 mg of liver tissue. Microsatellites were isolated using two different protocols. In the first (loci *SfoLav 103–177*, see succeeding discussion), genomic DNA was digested using *Sau*3A1. DNA fragments were amplified via polymerase chain reaction (PCR) with degenerate decamer primers and enriched via hybridization with $(TG)_{10}$ and $(TC)_{10}$ oligonucleotides on nylon (Hybon N +) membrane. Recovered enriched fragments were amplified by PCR using the degenerate primer and TA-cloned in pUC 18 plasmid with an *Eco*RI cut site. Sequencing was done using *M13F/M13R* or *T7/SP6* primers on an ABI377 (Applied Biosystems). A total of 2112 colonies were screened with 643 positive colonies identified. Of these, 611 were sequenced of which 107 were successful. We present eight of these markers that are polymorphic in *S. fontinalis*.

For loci *Sfo226Lav-Sfo371Lav* (see succeeding discussion), CA-enriched microsatellite libraries for *S. fontinalis* were prepared by Genetic Identification Services (GIS; http://www.genetic-id-services.com/) using magnetic bead capture technology (see Peacock *et al*. 2002). DNA from positive plasmids was isolated using modified mini alkaline-lysis with a PEG precipitation step (PRISM Sequenase Terminator Double-Stranded DNA Sequencing Kit Protocol, PRISM Technologies) and sequenced using standard universal primers on an ABI 3100 sequencer (Applied Biosystems) (SUCoF, Université Laval). Primers were selected using AMPLIFY (W.R. Engels, Genetics Department, University of Wisconsin). A total of 557 positive clones were returned and 237 of these were sequenced. One hundred seventy-one of these sequences were of sufficient quality for further microsatellite development. Nomenclature for all primers developed here followed conventions outlined by Jackson *et al*. (1998) *Sfo* (for *S. fontinalis*); locus number; *Lav* (for Université Laval).

Genomic DNA for microsatellite amplification was extracted using a column system (DNeasy Extraction Kit, QIAGEN) from individuals of the following salmonid species: *S. fontinalis* ($n = 8$), *Salvelinus alpinus* ($n = 4$), lake charr (*S. namaycush*, $n = 4$), lake whitefish (*Coregonus clupeaformis*, $n = 4$), rainbow trout (*Oncorhynchus mykiss*; $n = 4$), cutthroat trout (*O. clarkii*), Atlantic salmon (*Salmo salar*; $n = 4$). Genotyping in *S. fontinalis* was performed on a set of two hybrid families (Rupert River × Laval River brook charr) being used in a molecular mapping project for the addition of markers to our mapping families. Heterozygosity in our estimates was necessarily thus a function of polymorphism within these families, and so we only report polymorphism as the number of alleles detected rather than heterozygosity.

Initial PCR screens of amplificability for *S. fontinalis* alone were performed for all primers using approximately 30 ng genomic DNA in 11-μL reactions with 1.1 μL of buffer [100 mM of Tris-HCl, 900 mM of KCl (pH 9.0)], 0.5 pmol of each primer, 3.0 mM of dNTPs, 1.5 mM of $MgCl_2$ and 1 unit of *Taq* DNA polymerase. Amplified loci were run on 1.5% agarose gels with ethidium bromide staining ($n = 2$). For genotyping, microsatellite fragments were amplified using the above protocol with dNTPs being replaced by 0.38 mM of fluorescent dUTP-Tamra (Molecular Probe Industries) and 2.3 mM of dATP, dCTP, and dGTP. Genotyping PCR was performed initially at 1.5 mM $MgCl_2$ and using annealing temperatures estimated from primer sequence (+2 °C for A or T; +4 °C for C or G), but both parameters were altered in subsequent runs as appropriate to improve fragment quality and intensity. PCR was carried out in an GeneAmp PCR System 9700 thermocycler using a standard three-step PCR profile: 3 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at the specific annealing temperature for the microsatellite (see Table 1) and 45 s at 72 °C; and a final 7 min at 72 °C.

Table 1 Sequence information and reaction parameters for 35 microsatellite loci developed for use in the brook charr (*Salvelinus fontinalis*)

Locus	Size	T_a	$MgCl_2$	Repeat motif	Primer sequence (5′–3′)
Sfo103Lav	154	57	1.5	$(TG)_{11}$	F: AGCACTGGGGCTTTGTAGTC R: TACAGGCATCCAATCACCTG
Sfo106Lav	145	57	1.0	$(TGG)_2(TG)_{10}(TTTA)_3ACC(TTTA)_2$	F: CGACTCGAGGCCCAAATG R: GCCCCTGAACAAAGCAGTTA
Sfo107Lav	164	50	1.5	$(TG)_4CG(TG)_8$	F: GTCGGAATGTTCCTATGTGG R: CAGTCGCAGCTGTAAATGTG
Sfo112Lav	166	57	1.5	$(GA)_7CA(GA)_9N_{20}(GA)_{11}$	F: GTGGTAACACTTTCCGTGCC R: AGAACTGCAACGCTGCTG
Sfo114Lav	231	48	1.0	$(TG)_{13}$	F: CAGTGTGAGTTTGTGATGC R: CGTGAGTTCATGTCGACTGC
Sfo125Lav	184	50	1.0	$(AG)_{11}N_{12}(AG)_9$	F: GCGGGATTTATGTGGAGAGA R: TGTGGGCATGACAACTAACAG
Sfo170Lav	243	59	1.5	$(CA)_3GA(CA)_{10}$	F: GTGGCTCCATCATTTCAAG R: CAGTGTGTACATTGTCCTG
Sfo177Lav	441	55	1.5	$(TG)_{30}$	F: CGAATGTGGAGCTGAACTG R: GGGTATTTGTACAATGGGT
Sfo226Lav	389	60	1.2	$(TG)_{21}(CGTG)_{13}$	F: GAGGGCTAGAGACTAGCTTCAG R: GCAGTGGAACAAATACCCAG
Sfo227Lav	303	50	1.5	$(TG)_{14}CG(GCGT)_8$	F: GGGAAGAATGTTAGCCTGTG R: CTGAGGTGCTGCTGGATG
Sfo228Lav	262	54	1.5	$(CA)_{16}$	F: CAGGACGCCATATTGGGAG R: CCAGCCATGCCATGTTTG
Sfo233Lav	241	50	1.5	$(TG)_{14}$	F: GGCCTTGCTTATTATAGAC R: CCACCTATTGCTAGGTGC
Sfo235Lav	214	50	1.5	$(CACG)_7(CG)_3CACG(CA)_{23}AATG(CA)_4$	F: GATCAGTTTACAGATGAGC R: GACATGTAAAGTTGTGCC
Sfo241Lav	246	50	1.5	$(TC)_4(TG)_{16}(CGTG)_{14}$	F: CTCCATTAGAAAGGGTTTG R: CCAGTCTTTAGTCAACGC

Table 1 *Continued*

Locus	Size	T_a	$MgCl_2$	Repeat motif	Primer sequence (5′–3′)
Sfo242Lav	240	52	1.5	$(TG)_{35}$	F: CTTAAAGGACCCTGTGAAGG R: CATATACTCAAGTGTGATCC
Sfo244Lav	216	62	1.5	$(TG)_{21}CGTGCGTG(TGCGCG)_5$	F: GGTCAGTGCCAATACTATC R: CTAACAAAATGGCTACACG
Sfo247Lav	331	60	1.2	$(TG)_{14}$	F: GTGTTGTCAGGGAGCAACTG R: CACAAATGTTTGCGATATGACG
Sfo252Lav	206	60	2.0	$(CA)_{15}$	F: CATCGCTTCTCCAGCCATGAC R: CATGAATTATGAAGGTCAGTGC
Sfo257Lav	262	55	1.5	$(CA)_3(GC)_5(CACG)_{11}(CA)_{35}$	F: GGGCACATATTTCTACTGAC R: CTTTCCAGGCACTGCAAATG
Sfo261Lav	203	52	1.2	$(TG)_{17}(CG)_8$	F: GTATGGAGTATGGAAGAC R: CTTTATCCTCCTTCGGTTC
Sfo262Lav	269	60	1.5	$(TG)_{20}(CGTG)_7CGCG(CGTG)_2(CG)_3$	F: CCCATGTCAGTATTGGACTC R: CTTCATGGGCAGAATGGAC
Sfo266Lav	254	52	1.5	$TGCG(TG)_{13}N_{16}(TG)_3TGCG$	F: CTGGCAGCATTGTAAAGAAG R: CTGGGTGATTTGACGACC
Sfo269Lav	220	48	1.5	$(CA)_{28}$	F: GTAGATGAAACCTGATGG R: GTTCTATGGTCACATACTG
Sfo271Lav	389	50	1.5	$TGTC(TG)_9TCTG$	F: GAATTGACTAAATGCAGCC R: CAAATGTTTGCGATATGACG
Sfo275Lav	196	64	1.5	$(GT)_{21}(CG)_2(CGCT)_{12}(GA)_5$	F: CACTGCTGTAGTTGGTGGC R: GGTAGACCTGCTCTGAGAAC
Sfo289Lav	272	52	55	$(GT)_{30}(GCGT)_{16}(GT)_4$	F: CCTGGGTCACCATTGGCTTTC R: CACGTAGTCATGCAGACTAGC
Sfo290Lav	219	54	1.5	$(CT)_6(CGCA)_5(CA)_2(CGCA)_7(CA)_9CGCA(CA)_5$	F: GGTAACTGATGTTGCATTCAC R: CTCGACAACCCATAATCACG
Sfo292Lav	202	62	1.2	$(TG)_3A(TG)_{17}$	F: CCTTAGTCCCCTGTGCTTG R: CTGAGACCGCACTGGTACAC
Sfo305Lav	262	52	1.5	$(CACG)_{16}(CA)_{23}$	F: GGAGTGTGAATCTCAGGCTC R: CTCTCCATGCCTGTATAAGG
Sfo308Lav	343	60	1.5	$(TG)_{26}$	F: CAGCAATGGGGCTGAAGTAG R: GTCACTGTGTGAATCCTCC
Sfo320Lav	162	55	1.5	$(CA)_{17}$	F: GACCACTTCCACCTGATCCC R: CAAAGGGATACTGCTTATGC
Sfo327Lav	276	52	1.0	$(TG)_{17}TC(TG)_{51}$	F: GGTGACTGCCTGATAAAGC R: CATTCACATGCGCTTACGTG
Sfo329Lav	264	52	1.5	$(TG)_{38}$	F: GTACAAAGGTACTGAGGTC R: GAGAAAGTGAAAAGGCACC
Sfo333Lav	267	50	1.5	$(TG)_{59}(CGTG)_{14}$	F: GGAGAACACTCAGTGAGAAC R: GAGAGGAATCGGCTTGGC
Sfo334Lav	320	52	1.5	$(TG)_{58}G_4(TG)_{27}(CGTG)_8$	F: GGATTAACAGAAGGTTACTG R: CTTCGTATTCTTCATTGTGC
Sfo335Lav	219	57	1.0	$(TG)_{39}(CGTG)_{15}$	F: GGAGATTAGTGGGCTCATGC R: CTGGGACCCATCATTGTGGC
Sfo340Lav	227	57	1.5	$(TG)_{36}CG(CGTG)_8$	F: CTTCTGATCCTGCTCTTTG R: GGACCATGCTATTAGCATC
Sfo342Lav	357	64	1.0	$(TG)_{29}CG(TG)_{26}$	F: GCTGTCTTAGAAGTGATGTC R: CTGCCTGGCACAGTCATC
Sfo352Lav	272	52	1.5	$(CGCA)_9(CA)_{21}$	F: GACTGGTTTTCTAAGGCAG R: CTTAAAAGTTGCCACTGGG
Sfo361Lav	220	62	1.5	$(TG)_{27}$	F: CATAATCCATAGGCTAATCC R: GACTGACCACTGAGAATC
Sfo371Lav	305	60	2.0	$(CA)_{13}$	F: GCGATATGACGAAATACAATC R: GTTGTCAGGGAGCAACTGC

Size of the cloned fragment (*Sau* 3A1 digest/decamer amplification or magnetic bead capture technology/PEG precipitation size) is given in base pairs, annealing temperature (T_a) in °C and magnesium chloride concentration ($MgCl_2$) in mM. Repeat motif indicates the core repetitive sequence for the microsatellite. Microsatellite nomenclature follows Jackson *et al*. (1998).

Microsatellite fragments were separated in 8% polyacrylamide gels run at 80 W (~1900 V) for 1.0–1.5 h, scanned at 605 nm using an FMBIO II flatbed scanner (Hitachi Biosystems) and analysed in IMAGEANALYSIS (Hitachi Genetic Systems). Rox-500 standards (Applied Biosystems) were used to characterize fragment lengths.

A total of 111 microsatellites developed using the two protocols were pretested using agarose gels, 66 of which appeared to produce appropriate signal in *S. fontinalis*. Of the latter, a total of 41 microsatellite loci (eight microsatellites from the first protocol and 33 from the second) were polymorphic in at least one of the salmonid taxa tested (Table 1). Successful fragment amplification and microsatellite polymorphism was relatively higher in *Salvelinus* and the putative sister taxa *Oncorhynchus* than in the two representative species of the more distantly related *Salmo* and *Coregonus* (Table 2) (see Crespi & Fulton 2004). Thirty-six loci were polymorphic in *S. fontinalis* (37 including

Table 2 Cross-amplification of *SfoUL* primers from Table 1 in salmonid fish

Locus	*Safo* (*n* = 8)	*Saal* (*n* = 4)	*Sana* (*n* = 4)	*Onmy* (*n* = 4)	*Oncl* (*n* = 4)	*Cocl* (*n* = 4)	*Sasa* (*n* = 4)
Sfo103Lav	154–158* (2)	143* (1)	145–147* (2)	148–150* (2)	151* (1)	na	na
Sfo106Lav	148* (1)	152* (1)	145–147* (2)	na	149* (1)	na	na
	182–190* (2)	162* (1)			155* (1)		
Sfo107Lav	167–195* (4)	178* (1)	167– 193* (2)	na	na	na	na
Sfo112Lav	176–206* (2)	140–168* (3)	178* (1)	na	184* (1)	170* (1)	na
Sfo114Lav		97* (1)	na	na	96* (1)	na	na
		195* (1)	198* (1)			300* (1)	
	270–307* (3)	250* (1)			308* (1)		
Sfo125Lav	188– 200* (5)	274* (1)	na	na	na	na	na
Sfo170Lav	240–244* (2)	236† (1)	238–248† (3)	244–248† (2)	230† (1)	244–250† (2)	na
		250† (1)					
Sfo177Lav	314* (1)	228* (1)	na	292* (1)	314* (1)	na	na
	410–420* (2)	316* (1)					
Sfo226Lav	373–381* (3)	383–407§ (3)	340§ (1)	333* (1)	418–430§ (2)	na	na
Sfo227Lav	229–283* (4)	205* (1)	260* (1)	294* (1)	270* (1)	312* (1)	166–170* (2)
		280* (1)	275* (1)			340* (1)	
Sfo228iLav	296–306* (2)	260* (1)	210* (1)	255* (1)	260‡ (1)	260§ (1)	250* (1)
Sfo228iiLav	136–142* (2)	166–176* (2)					
Sfo233Lav	234–244* (4)	252–256* (2)	428–434* (2)	238–264* (3)	248–256* (3)	na	na
Sfo235Lav	197–209* (3)	na	156–162* (2)	157* (1)	170* (1)	243* (1)	na
Sfo241Lav	273–305* (2)	225* (1)	na	219–225* (2)	217–227* (2)	na	na
Sfo242Lav	206–268§ (4^n)	178* (1)	195–227‡ (3)	184* (1)	172* (1)	na	na
Sfo244Lav	196–204* (6)	343* (1)	na	na	383–391* (4)	na	na
Sfo247Lav	333–351§ (2)	351* (1)	313–363§ (4)	na	351* (1)	na	na
Sfo252Lav	165‡ (1)	165‡ (1)	na	165‡ (1)	165‡ (1)	165‡ (1)	na
	255–343‡ (3)						
Sfo257Lav	318† (1)	350–390§ (2)	170† (1)	156–170‡ (4)	158‡ (1)	154–160* (2)	152–154* (2)
	438–448‡ (4)						
Sfo261Lav	218–224* (3^n)	220–228* (3)	na	170* (1^n)	218–220* (2^n)	na	na
Sfo262Lav	299–321* (8)	345–369* (4)	na	327–347* (4)	na	295–299* (4)	na
Sfo266Lav	258–292* (4)	237* (1)	215§ (1)	270–306§ (4)	301* (1)	na	na
Sfo269Lav	221–243* (3)	237‡ (1)	212† (1)	221–263* (4)	207–267† (4)	na	na
		265† (1)					
Sfo271Lav	402* (1)	410–420‡ (2)	402* (1)	402* (1)	402* (1)	402* (1)	402* (1)
Sfo275Lav	196–250* (4)	161–167* (2)	148–152* (2)	125–129* (2)	129* (1)	na	na
Sfo289Lav	201–245‡ (5)	267–305§ (3)	na	163–171‡ (3)	196–234* (4)	na	na
Sfo290Lav	241–254* (5)	178* (1)	176* (1)	280–290§ (2)	226–258‡ (2^n)	na	na
Sfo292Lav	195–253* (5)	224–230* (2)	247–267* (2)	na	240–268* (2)	235–237* (2)	213–257‡ (2)
Sfo305Lav	394* (1)	392* (1)	396–428* (2)	448–484* (2)	364–394* (2)	430* (1)	na
	352–378* (4)						
Sfo308Lav	355–379* (4)	348–352§ (2)	na	348–374* (3)	435–449* (2)	na	na
Sfo320Lav	152–168* (4)	108* (1)	na	140–150* (3)	134–182* (2)	143* (1)	na
		179* (1)					
Sfo327Lav	na¶	na	na	181–189* (2)	319* (1)	na	na
Sfo329Lav	na¶	185– 197* (2)	290* (1)	133–135* (2)	na	161* (1)	290* (1)

Table 2 *Continued*

Locus	*Safo* (*n* = 8)	*Saal* (*n* = 4)	*Sana* (*n* = 4)	*Onmy* (*n* = 4)	*Oncl* (*n* = 4)	*Cocl* (*n* = 4)	*Sasa* (*n* = 4)
Sfo333Lav	212* (1) 230† (1)	212* (1) 258* (1)	na	202–212* (2)	212* (1) 258* (1)	212* (1)	na
Sfo334Lav	na¶	238–296* (7)	165– 193* (5)	na	255–259* (2)	402* (1)	na
Sfo335Lav	150–156* (2)	na	na	na	174–247§ (2)	228–250* (2)	na
Sfo340Lav	162–240† (3^{n})	na	na	154–162* (2)	162* (1)	na	na
Sfo342Lav	348–404§ (4)	na	na	na	na	na	na
Sfo352Lav	306–310§ (3^{n})	276–280* (2)	282–298§ (2)	207–231‡ (4^{n})	229–323† (2)	228–268* (3)	na
Sfo361Lav	170–188* (2)	na 137* (1)	163* (1) 137–149* (2)	167* (1) 137–149* (2)	167* (1)	na	na
Sfo371Lav	342–438‡ (3)	318§ (1)	313–353§ (2)	316–348‡ (5)	na	330–340† (4)	344–384§ (3)
n	37	15	14	21	16	7	4

Safo, *S. fontinalis*; *Saal*, *S. alpinus*; *Sana*, *S. namaycush*; *Onmy*, *O. mykiss*; *Oncl*, *O. clarkia*; *Cocl*, *C. clupeaformis*; *Sasa*, *S. salar*; *n*, number of individuals.

Fragments amplified by each primer pair are indicated as fragment size$^{\text{fragment quality}}$ (number of alleles). The first number refers to the length of amplified fragments (in base pairs). Symbols refer to the quality of the generated fragments: *clear, strong amplification; †clear fragments, weak amplification; ‡strong fragments, smeary; §weak fragments, smear; ¶unacceptable product in *S. fontinalis*.

Numbers in parentheses indicate the number of fragments. Possible null alleles are indicated by^{n}; ineffective amplification is represented by 'na', *n* (italics; bottom) is the number of polymorphic microsatellite loci by species.

Sfo228Lav was duplicated, doubly polymorphic locus in *S. fortinlas* and is therefore counted twice for the total number of polymorphic loci for that species.

the simultaneous polymorphism at the homologous and homeologous loci of *Ssa228Lav*), 15 in *S. alpinus* and 14 in *S. namacush* (Table 2). Three loci (*Sfo327Lav*, *Sfo329Lav*, and *Sfo334Lav*) generated signal of the appropriate size range for the cloned fragment (from sequencing), but of insufficient quality in the genotyping reactions. Two loci (*Sfo271Lav* and *Sfo333Lav*) were monomorphic in *S. fontinalis* but not in the other salmonids tested. In *Oncorhynchus*, polymorphism was high with 21 polymorphic loci amplifying in *O. mykiss* and 16 in *O. clarkii*. Polymorphism was low by comparison in *C. clupeaformis* (seven loci) and *S. salar* (five loci). Three screened loci (*Sfo327Lav*, *Sfo329Lav* and *Sfo334Lav*) did not generate a signal of sufficient quality in *S. fontinalis* to be useful.

Thirteen *SfoLav* loci appeared to be duplicated (tetraploid, expressing both the homologous and homeologous loci; see Allendorf & Thorgaard 1984) but at least one of the duplicate loci was monomorphic for all such duplicates save *Sfo228iLav* and *Sfo228iiLav* in *S. fontinalis*. Most evidence for duplication occurred in *Salvelinus*, suggesting that the conservation of homologous loci was higher in the source genus (Table 2). One locus, *Sfo335Lav*, appeared to contain null alleles — there was a marked predominance of homozygotes and nonamplifying individuals.

The relatively few microsatellite loci for the brook charr has somewhat hampered the collection of genetic information for this species, forcing workers to use nonspecific markers fortunately cross-amplifying in brook charr. Our work expands the range of microsatellites available to geneticists employing brook charr models for the purposes of molecular ecology, paternity analysis and genetic variance estimation in addition to cross-amplificative capacity in other species of salmonids.

Acknowledgements

We would like to thank A. Crête-Lafrèniere, M. Matsuoka, L. Papillon, C. Lippé and R. St-Laurent for their assistance in the screening and designing of the microsatellites used here. This work was funded by the NSERC Genomic program and is a contribution to the Canada Research Chair in Conservation Genetics of Aquatic Resources (LB).

References

Allendorf FW, Thorgaard GH (1984) Tetraploidy and the evolution of salmonid fishes. In: *Evolutionary Genetics of Fishes* (Turner BJ), pp. 1–53. Plenum Press, New York.

Angers B, Bernatchez L (1996) Usefulness of heterologous microsatellites obtained from brook charr, *Salvelinus fontinalis* Mitchill, in all Salvelinus species. *Molecular Ecology*, **5**, 317–319.

Angers B, Bernatchez L, Angers A, Desgroseillers L (1995) Specific microsatellite loci for brook charr (*Salvelinus fontinalis* Mitchill) reveal strong population subdivision on a microgeographic scale. *Journal of Fish Biology*, **47** (Suppl A), 177–185.

Brunner PC, Douglas MR, Osinov A, Wilson CC, Bernatchez L (2000) Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **55**, 573–586.

Castric V, Bonney F, Bernatchez L (2001) Landscape architecture and heirarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution*, **55**, 1016–1028.

Crespi BJ, Fulton MJ (2004) Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. *Molecular Phylogenetics and Evolution*, **31**, 658–679.

Danzmann RG, Morgan RP, Jones MW, Bernatchez L, Ihssen PE (1998) A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook trout (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. *Canadian Journal of Zoology*, **76**, 1300–1318.

Gislason D, Ferguson MM, Skulason S, Snorrason SS (1999) Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic charr (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 2229–2234.

Jackson TR, Ferguson MM, Danzmann RG *et al*. (1998) Identification of two QTL influencing upper temperature tolerance in rainbow trout (*Oncorhynchus mykiss*). *Heredity*, **80**, 143–151.

Jonsson B, Jonsson N (2001) Polymorphism and speciation in Arctic charr. *Journal of Fish Biology*, **58**, 605–638.

Peacock MM, Kirchoff VS, Merideth SJ (2002) Identification and characterization of nine polymorphic microsatellite loci in the North American pika. Ochotona princeps. *Molecular Ecology Notes*, **2**, 360–362.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring, New York.