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TECHNICAL NOTE

Development of ten new EST-derived microsatellites in Atlantic cod (*Gadus morhua* L.)

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Abstract Ten polymorphic microsatellite markers were developed from approximately 1,300 expressed sequence tags (ESTs) of Atlantic cod (*Gadus morhua* L.). Thirty two primer pairs were designed for EST sequences containing perfect di- tri- tetra- and pentanucleotide motifs and characterised in 96 unrelated fish. Ten markers were successfully amplified with number of alleles from 2 to 13 per locus and observed and expected heterozygosity ranging from 0.03 to 0.69 and 0.03 to 0.74, respectively. Loci *Gmo*-C131, C132 and C136 deviated from Hardy-Weinberg equilibrium. Genetic linkage disequilibrium analysis between all pairs of the loci showed significant departure from the null hypothesis between loci *Gmo*-C131 and *Gmo*-C132 and C128 and *Gmo*-C133. The gene identity was determined at five of the loci, confirming the associated microsatellites as Type I markers. The new microsatellites reported in this work can be used for conservation and enhancement of wild stocks for commercial harvesting.

Keywords Atlantic cod · Expressed sequence tag · Microsatellite · Type I marker

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The Atlantic cod (*Gadus morhua* L.) is very common along the entire Norwegian coast. Based on their life history characteristics and migration patterns, Atlantic cod in Norwegian and adjacent waters have traditionally been divided into three stocks, i.e. the northeast Arctic cod (NEAC), the Norwegian coastal cod (NCC) and the North Sea cod (NSC). Local populations exist in many fjords that do not interact extensively with populations living in the open sea (Fevolden and Sarvas 2005; Sarvas and Fevolden 2005a; Sarvas and Fevolden 2005b). Microsatellite markers have been successfully applied in studies of population structure of NEAC (Beacham et al. 2002; Bentzen et al. 1996; Ruzzante et al. 1996; Ruzzante 1998) and NCC (Hutchinson et al. 2001; Knutsen et al. 2003; Knutsen et al. 2004; Nielsen et al. 2001; Nielsen et al. 2003). However, Atlantic cod has a narrow genetic base and more genetic markers should be developed. Recently, we have published a number of microsatellite markers derived from both genomic library (Wesmajervi et al. 2006; Westgaard et al. 2006) and expressed sequence tags (ESTs) (Stenvik et al. 2006). In contrast to genomic microsatellites, EST-derived microsatellite markers have the potential to be functional markers (Varshney et al. 2005). In this paper we report the development of 10 EST-derived microsatellite loci from Atlantic cod. The new microsatellites presented in this work and those previously reported (Brooker et al. 1994; Jakobsdottir et al. 2006; Miller et al. 2000; O'Reilly et al. 2000; Wesmajervi et al. 2006; Westgaard et al. 2006; Stenvik et al. 2006) can be used for

Table 1 Ten microsatellite loci isolated from Atlantic cod (*Gadus morhua* L.) and PCR primers that were employed to screen 96 individuals. Significance level was adjusted following sequential Bonferroni correction

Locus	Repeat motif	Primer sequence (5′- 3′)	*A*	Allele size range (bp)	H_o	H_E	F_{IS}	GeneBank accession no.
Gmo- C127	CA	**F-CTGCTCTACGAGGCCATCACC R-GTTTCTTTGCATCAATTTGGCTTCACAC	13	217–249	0.69	0.74	0.0747	DQ884952
Gmo- C128	CGG	**F-ATGCCGACGAGGCCAAGGATG R-GTTTCTTAGCCGCCCTCGTTGTTGTAGC	4	172–195	0.03	0.03	–0.0071	DQ884953
Gmo- C129	TCC	**F-CCAGCTCAAGCAGCATTTCTA R-GTTTCTTAATGACTGTTGCTTCGACACG	2	99–105	0.04	0.04	–0.0162	DQ884954
Gmo- C131	CTC	**F-CTCCCAAGTAGTGCGTCTTC R-GTTTCTTAACGAATCTGTGATCCTAAACA	2	123–126	0.00	0.44	*1.0000	DQ887771
Gmo- C132	CTCCC	**F-TTCCATCGTCCACCGCAAATG R-GTTTCTTTTCGCGCAGAGCTGAAGTCGT	2	100–104	0.01	0.44	*0.9762	DQ884955
Gmo- C133	CGG	**F-TGCCGACGAGGCCAAGGATG R-GTTTCTTAGCCGCCCTCGTTGTTGTAGC	3	185–194	0.04	0.04	–0.0121	DQ884956
Gmo- C134	AGA	**F-TCTGGCCCGTGCTGGTAAAG R-GTTTCTTGCGCCGGTTGTACTGGATG	6	238–292	0.37	0.34	–0.0963	DQ884957
Gmo- C135	AC	**F-GCAAAGCCTTCGGGCAAGAT R-GTTTCTTCAGTTGAAGTTCATAATATAGA	3	127–131	0.20	0.21	0.0908	DQ884958
Gmo- C136	TCC	**F-ACGAGGAGCAGTTCTAGCA R-GTTTCTTAACTTCATCTCGAATGACTGT	2	114–117	0.00	0.05	*1.0000	DQ884959
Gmo- C138	CA	**F-CCATCCCAAATGAGATTATC R-GTTTCTTTCCTGCGGAGTGAGGGATTCT	4	125–133	0.24	0.22	–0.0737	DQ884960

($P < 0.05$). **fluorescent dye labelled primer. F: forward; R: reverse

A—number of observed alleles. H_o—observed heterozygosity. H_E—expected heterozygosity. F_{IS}—inbreeding coefficient, *depicts significant departure from Hardy-Weinberg equilibrium. Clone sequences are available on GenBank (Accession numbers DQ884952 to DQ884952 and DQ887771)

conservation and enhancement of wild stocks for commercial harvesting.

In an ongoing genomics program, we are generating ESTs from cDNA libraries of different developmental stages of Atlantic cod (unpublished results). Approximately 1,300 ESTs from Cod embryos were investigated for the presence of microsatellite motifs using Tandem Repeats Finder software (Benson 1999). A set of 32 flanking primer pairs were designed for EST sequences containing di- tri- tetra- and pentanucleotide motifs using Primerselect (DNASTAR). For fluorescent detection the forward primers were dye labelled (PET, NED, VIC or 6-FAM) (Applied Biosystems; AB). A PIG-tail sequence 5′-GTTTCTT (Brownstein et al. 1996) was included in the reverse primers (Table 1). Genomic DNA from 96 unrelated individuals was analysed to evaluate the efficiency of amplification and polymorphism of the markers by polymerase chain reaction (PCR) in a 2720 Thermal Cycler (AB). The amplification reaction was carried out in 10 μl volume containing 10–50 ng template, 1 × PCR-buffer (10 mM Tris–HCl; pH 8.8 at 25°C, 50 mM KCl and 0.1% Triton X-100), 2.25 mM Mg^{2+}, 400 μM each dNTP, 100 nM each primer and 0.4 U Dynazyme® II polymerase. Thermal cycling conditions were: 5 min at 94°C, followed by 30 amplification cycles at 94°C for 20 s, 58°C for 20 s and 72°C for 30 s and then a final extension at 72°C for 10 min. Genotyping was performed using a 3130*xl* Genetic Analyzer (AB). Data were collected automatically and sized with GeneMapper software (AB). Out of 32 primer pairs only 10 were readily scorable and polymorphic (Table 1). Statistical calculations were performed using the program Genepop version 3.4 (Raymond and Rousset 1995). The number of alleles per locus (*A*), observed heterozygosity (H_o), expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}) were calculated. The number of alleles varied from 2 to 13 per locus and the observed- and expected heterozygosity ranged from 0.03 to 0.69 and 0.03 to 0.74, respectively (Table 1). The loci were further tested for both Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium and significance levels were determined after 100 batches of 5,000 interactions each. Three of the loci (*Gmo*-C131, C132 and C136) deviated from HWE after sequential Bonferroni correction (Rice 1989), probably due to the small sample size, genetic drift and the presence of null alleles. Two of 45 pairwise exact tests rejected genotypic equilibrium between loci (*Gmo*-C131 and *Gmo*-C132 and C128 and *Gmo*-C133) after sequential Bonferroni correction. BLAST analysis gave highly significant hits for five loci (Table 2).

Table 2 Annotation of gene-associated microsatellites based on BLAST searches against the NCBI GenBank non-redundant database. The putative location of the microsatellite within the gene is indicated and the accession no. of the matching sequence is given

Locus	TBLASTX similarity match	Gene region	Species	GenBank accession no.
Gmo-C128	hyperosmotic glycine rich protein mRNA	3′UT	Salmo salar	AF533016
Gmo-C129	vitellogenin B (VtgB) mRNA	3′UT	Melanogrammus aeglefinus	AF284034
Gmo-C132	beta actin mRNA	3′UT	Acanthopagrus schlegelii	AY491380
Gmo-C133	hyperosmotic glycine rich protein mRNA	3′UT	Salmo salar	AF533016
Gmo-C136	vitellogenin B (VtgB) mRNA	3′UT	Melanogrammus aeglefinus	AF284034

These represent well-characterized genes thus defining the associated microsatellites as Type I markers, all located to the 3′UT region of the genes. In conclusion, these 10 polymorphic microsatellite loci provide useful tools for studies of population genetics, reproductive ecology and for constructing linkage maps of Atlantic cod.

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References

Beacham TD, Brattey J, Miller KM, Le KD, Withler RE (2002) Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. Ices J Mar Sci 59:650–665

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Bentzen P, Taggart CT, Ruzzante DE, Cook D (1996) Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can J Fish Aquat Sci 53:2706–2721

Brooker AL, Cook D, Bentzen P, Wright JM, Doyle RW (1994) Organization of microsatellites differs between mammals and cold-water teleost fishes. Can J Fish Aquat Sci 51:1959–1966

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by tag DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20:1004–1010

Fevolden SE, Sarvas T (2005) Atlantic cod (*Gadus morhua*) a new species in aquaculture in Norway–knowledge of genetic structuring in natural populations must be implemented in the design of breeding programs. Aquaculture 247:13

Hutchinson WF, Carvalho GR, Rogers SI (2001) Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. Mar Ecol-Prog Ser 223:251–260

Jakobsdottir KB, Jorundsdottir OD, Skirnisdottir S, Hjorleifsdottir S, Hreggvidsson GO, Danielsdottir AK, Pampoulie C (2006) Nine new polymorphic microsatellite loci for the amplification of archived otolith DNA of Atlantic cod, *Gadus morhua* L. Mol Ecol Notes 6:337–339

Knutsen H, Andre C, Jorde PE, Skogen MD, Thuroczy E, Stenseth NC (2004) Transport of North Sea cod larvae into the Skagerrak coastal populations. Proc R Soc Lond B-Biol Sci 271:1337–1344

Knutsen H, Jorde PE, Andre C, Stenseth NC (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. Mol Ecol 12:385–394

Miller KM, Le KD, Beacham TD (2000) Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Mol Ecol 9:238–239

Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P (2003) Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. Mol Ecol 12:1497–1508

Nielsen EE, Hansen MM, Schmidt C, Meldrup D, Gronkjaer P (2001) Fisheries–Population of origin of Atlantic cod. Nature 413:272

O'Reilly PT, Canino MF, Bailey KM, Bentzen P (2000) Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. J Fish Biol 56:1074–1086

Raymond M, Rousset F (1995) Genepop (Version-1.2)–population-genetics software for exact tests and ecumenicism. J Heredity 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Ruzzante DE (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Can J Fish Aquat Sci 55:1–14

Ruzzante DE, Taggart CT, Cook C, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: Microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53:634–645

Sarvas TH, Fevolden SE (2005a) Pantophysin (Pan I) locus divergence between inshore v. offshore and northern v. southern populations of Atlantic cod in the north-east Atlantic. J Fish Biol 67:444–469

Sarvas TH, Fevolden SE (2005b) The scnDNA locus Pan I reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. Fish Res 76:307–316

Stenvik J, Wesmajervi MS, Fjalestad KT, Damsgard B, Delghandi M (2006) Development of 25 gene-associated microsatellite markers of Atlantic cod (*Gadus morhua* L.). Mol Ecol Notes 6:1105–1107

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55

Wesmajervi MS, Tafese T, Stenvik J, Fjalestad KT, Damsgard B, Delghandi M (2007) Eight new microsatellite markers in Atlantic cod (*Gadus morhua* L.) derived from an enriched genomic library. Mol Ecol Notes 7:138–140

Westgaard JI, Tafese T, Wesmajervi MS, Stenvik J, Fjalestad KT, Damsgard B, Delghandi M (2006) Identification and characterisation of thirteen new microsatellites for Atlantic cod (*Gadus morhua* L.) from a repeat-enriched library. Conserv Genet doi.org/10.1007/s10592–006–9200–3