the conservation genetics of *Dasyurus* species (K. B. Firestone *et al*. unpublished).

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Microsatellite markers for the hake *Macruronus magellanicus* amplify other gadoid fish

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The long-tail hake *Macruronus magellanicus* is a demersal–pelagic species that constitutes the most abundant fish resource in the southwestern Atlantic (Prenski *et al*. 1996). However, the fisheries have only recently developed and stock identification is required before large-scale exploitation occurs. However, ecological (R. Gonzalez, B. Prenski, personal communication) and morphological (A. Giussi, personal communication) data suggest stock differentiation in Patagonian gulfs. Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP), the only genetic data available, detected no differentiation among coastal and offshore samples (M. E. D'Amato & G. R. Carvalho, unpublished), indicating a need to develop more polymorphic markers such as microsatellites.

DNA was extracted from fresh muscle tissue according to Corach (1991) or from ethanol-preserved samples by saltation (Brufford *et al*. 1992). Prior to microsatellite isolation, an enrichment procedure using random amplified polymorphic DNA (RAPD)–PCR similar to that of Cifarelli *et al*. (1995) was employed. Amplification of 20–100 ng of DNA was performed in a 15 μL final volume with 0.2 mM each dNTP, 2 mM $MgCl_2$, 0.5 U *Taq* polymerase (BioLine), and 5 pmols of one or two RAPD primers (Kit A, Operon Technologies Inc). Reactions were run in a Hybaid Omnigene Thermocycler, at 94 °C for 3 min, 40 cycles at 93 °C for 50 s, 37 °C for 1 min, 72 °C for 1 min; 72 °C for 10 min. Half-volume (7.5 mL) of all 60 RAPD reactions was run in 1% agarose gels in TBE buffer, and transferred to nylon membranes by Southern blot (Sambrook *et al*. 1989). Repetitive elements were detected by hybridization to [γ^{32}P]-dATP 5′ end-labelled $(AC)_{12}$ $(AG)_{12}$ $(CAG)_7$ $(TAC)_8$ $(AAC)_8$ $(GTG)_8$ $(AAG)_8$ $(AAT)_8$ $(AAAT)_6$ $(AAAC)_6$ $(GATA)_{10}$ $(GACT)_6$ $(GACA)_6$ and $(GTAT)_6$. After autoradiography, 1 μL of the remaining PCR reactions which showed positive bands was cloned into pGEM -T Easy Vector (Promega) and INVa F′ (Invitrogen® *Escherichia coli* cells were transformed by the TFB–DMSO method as described in Sambrook *et al*. (1989). Approximately 2200 colonies were transferred to nylon membranes and probed with the same [γ^{32}P]-labelled oligos. Depending on the RAPD primers used, the ratio of positive to negative colonies ranged between 1:9 and 1:33. A total of 38 colonies was sequenced with universal M13 Cy5-labelled primers (T7 Sequenase version 2.0, Amersham) on an ALF*express*™ automatic sequencer (Pharmacia). Microsatellites were found in 30 clones. Ten of these microsatellites, however, were complex or long (150 bp or more) dinucleotide repeats that were discarded because these primers are also intended for ancient DNA analysis, where long DNA tracts are more difficult to amplify. Two clones were found to contain two different microsatellites (Mm 4–2, and Mm 19–1 g), while Mm 14–1 contains three.

PCR reactions were performed in 10 μL volumes containing 0.2 mM dNTPs, 2 mM $MgCl_2$ (except Mm 9–2, 1.5 mM $MgCl_2$), 0.12 μM each primer. Extensive optimization was carried out for all primer pairs on *M. magellanicus* and several other gadoid species. Optimal conditions are described in Tables 1 and 2 by the following numerical system: (1) initial denaturing 97 °C for 30 s, followed by five cycles at 96 °C for 30 s, 60 °C for 40 s, 10 cycles at 96 °C for 20 s, 55 °C for 40 s, 25 cycles at 96 °C for 20 s, 50 °C for 30 s, and a final extension step at 72 °C for 1 min; (2) as (1), but five cycles at 65 °C followed by 30 at 60 °C; (3) 94 °C for 1 min, followed by five cycles at 94 °C for 45 s, 52 °C for 45 s, 72 °C for 45 s, followed by 30 cycles at 94 °C for 45 s, 50 °C for 45 s, 72 °C for 45 s, final extension at 72 °C for 3 min; (4) as (3), annealing temperatures: 54–52 °C; (5) as (3), annealing temperatures 46–48 °C.

Table 1 Microsatellite loci from *Macruronus magellanicus*. PCR conditions are indicated in the text. GeneBank Accession nos are indicated below each locus

Locus	Primers 5′–3′	Repeat sequence	T_m (PCR condition)	Alleles	Size range (bp)	H_O (no. of individuals)	H_E
Mm 110–8	F: CATGGTCGGGGAAATAGAGGG	$(CA)_{12}$	60 °C (1)	15	111–155	0.59 (41)	0.59
AF121788	R: CTACCAACGGATGAGCCAAAC						
Mm 110–13	F: CATGGAAGTGATTCATCTCTG	$(CA)_6CT(CA)_9$	60 °C (1)	22	101–151	0.56 (41)	0.65
AF121787	R: TTCGGCATGTACTCACTTTGC						
Mm 5–4	F: AACTCAAGTAACCCACAAAC	$(TGTA)_{11}$	60 °C (1)	12	207–257	0.61 (41)	0.83
AF121794	R: GAACCGCTCATCCAAACAAC						
Mm 14–1(T3)	F: ATCTAGTCTCTGTGCTGGCA	$(TAA)_8$	46–48 °C (5)	9	98–125	0.825 (38)	0.815
AF121786	R: TTTTCACTATTCCTTGTCCCA						
Mm 14–1(T4)	F: ACTGCACTCTGGACTGGGAC	$(GATA)_7$	60 °C (1)	9	99–127	0.625 (40)	0.64
AF121786	R: TTGAACTGAACCATAAATGCC						
Mm 18–1	F: GTTATGGAAACAAAATGTGCG	$(TTC)_4$	65 °C (2)	3	104–110	0.10 (39)	0.099
AF121790	R: TAGAGGTGACCGTCTTTTAT						
Mm 18–5	F: AGAGTTCATCCGATTTCACCG	$(CAG)_4$	60 °C (1)	2	96–99	0.395 (38)	0.447
AF121791	R: AATCTTCCCATTAGTATTCACGC						
Mm 17–4	F: CCATCAAGATATAATTACGCTG	$(ATCT)_{15}$	60 °C (1)	24	192–272	0.973 (37)	0.955
AF121789	R: GCTACTGTATGTATATGTAGCCGT	$ATAT(ATC)_{14}$ $(AT)_3CTATGT$ $ATCT(A)_6$					
Mm 19–1 g-1	F: TTAATGGCGGTAAGCGTGGC	TAAATACA	60 °C (1)	2	138–146	0.09 (22)	0.34
AF121792	R: GCCCGCATTTTACATTTCCC	$(TAAA)_5GCACA$ $(TAAA)_2$ TATATAAA					
Mm 19–1 g-2	F: AAGAAGAAGAGATGGGAGCG	$(GA)_{11}$	60 °C (1)	7	94–110	0.468 (32)	0.75
AF121792	R: CTTTATTCTGGCGGAGGACG						
Mm 422	F: TGCTCCTTAGTACTAAAACGC	$(TA)_9$	60 °C (1)	5	132–140	0.28 (39)	0.356
AF121793	R: TATTTTCATTTCAATACAGAGG						
Mm 9–2	F: GGTGAGTTCACCGATGTCGC	$(CA)_{20}$	65 °C (2)	8	122–142	0.72 (39)	0.878
AF121795	R: TGGCTAGCTAGACGCGATGT						

T_m = temperature of melting; H_O = observed heterozygosity; H_E = expected heterozygosity.

Table 2 Cross-species amplification with *Macruronus magellanicus* microsatellite primers. The table shows only results with clear bands and no stuttering. PCR conditions are indicated in the text

Primer	Species	PCR conditions	Allele size range in bp (no. of alleles)	*N*
Mm 110–8	*M. novozelandiae*	1	122–146 (6)	10
	Melanogrammus aeglifinus	4	160 (1)	3
	Merlangius merlangus	4	108 (1)	6
	Micromesistius poutasou	3	108–114 (3)	3
Mm 110–13	*M. novozelandiae*	1	103–119 (4)	10
	Gadus morhua	5	162 (1)	6
	Pollachius virens	3	109–123 (2)	3
	Trisopterus esmarkii	3	109–111 (2)	2
	M. merlangus	3	109–111 (2)	5
	Gadiculus argenteus	3	107–109 (2)	3
Mm 14–1(T4)	*M. novozelandiae*	1	100–124 (4)	6
	Molva molva	3	236 (1)	5
Mm 18–1	*M. novozelandiae*	1	107 (1)	4
	T. esmarkii	3	109–111 (2)	2
Mm 18–5	*M. novozelandiae*	1	91–94 (2)	4
Mm 9–2	*M. novozelandiae*	1	130–142 (6)	10
	M. merlangus	3	102 (1)	6
Mm 19-g1	*M. novozelandiae*	1	138–146 (2)	4
Mm 19–1 g2	*M. aeglifinus*	3	135 (1)	6

T_m = temperature of melting; *N* = sample size.

PCR products were run in ALF*express*™ along with size markers obtained as in Van Oppen *et al*. (1997).

Polymorphism was tested in a sample of 40 individuals from San Matias Gulf, northern Patagonia (Table 1). Only loci 19–1 g1 and 19–1 g2 deviated from Hardy–Weinberg (H–W) expectations (Fisher's exact test $P = 0.0004$ and 0.0007, respectively) after sequential Bonferroni correction (Rice 1989), arising possibly from population substructure, selection, inbreeding, sampling, or null alleles. No linkage disequilibrium between loci was detected after applying sequential Bonferroni (Rice 1989) correction.

Primers were also tested on other gadoid species listed in Table 2. The hoki *Macruronus novozelandiae* showed the highest level of polymorphism among the tested gadoids. Although sample sizes are low, some of these primers (Mm 110–8, 110–13 and 9–2) seem promising as population markers in several other gadoid species, as well as for evolutionary studies in this family.

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Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae)

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Fraxinus excelsior (L.) is an anemophilous (wind-pollinated) deciduous tree, distributed throughout Europe and Asia Minor. Its wood is still favoured by European foresters, as indicated by improvement and plantation programmes. These programmes rely increasingly on identification tools such as molecular markers in order to reduce ambiguities and to identify accurately elite material to be propagated.

To complete the few existing microsatellite loci (Brachet *et al*. 1999), this study reports the characterization of 10 new microsatellite markers in *Fraxinus excelsior* (L.) and shows their potential for further use in various species of the *Oleaceae* family, which contains the important forest genus *Fraxinus* as well as the olive genus *Olea* and many ornamentals.

Sixteen elite trees, from seven Irish and one French provenance, have been used for this study. Fourteen species from the genus *Fraxinus* and 11 *Oleaceae* species (one to six individuals of each species) were tested for cross-specific and cross-genera amplification. DNA extraction was performed on mature leaves of *Fraxinus excelsior* according to a rapid protocol of DNA extraction (Lefort & Douglas 1999). Young leaves from other *Fraxinus* and *Oleaceae* species were processed with the DNeasy Plant Mini Kit 50 (Qiagen).

A microsatellite-enriched library of *Fraxinus excelsior* was produced with a method described previously (Edwards *et al*. 1996). Eighteen single clones chosen at random were purified (Wizard Plus Miniprep DNA Purification System, Promega, Madison, WI, USA) and sequenced with the use of the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase FS (Perkin-Elmer Applied Biosystems). Sequence analysis was performed with an automated DNA sequencing system (Applied Biosystems 373 DNA Sequencer). Twenty-one microsatellite loci were obtained from these 18 clones but not all sequences were suitable for design of amplification primers. Twelve primer sets for PCR were designed empirically from the DNA sequences of 12 loci and 10 are shown here (Table 1). PCR amplifications for population screenings were conducted under the following conditions: 50 (one reaction included 75 mM Tris-HCl pH 9.0, 50 mM KCl, 1.3–2.5 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 0.001% gelatine, 62.5 μM dNTPs each (Biofinex, Praroman, Switzerland), 0.4–1 μM forward and reverse primer, 1.5 U AmpliTaq polymerase (Perkin-Elmer, Foster City, CA, USA) and 5–50 ng of DNA template. After an initial denaturation step of 5 min at 96 °C, the PCR consisted of 28–35 cycles with 1 min at 94 °C, 1 min at the appropriate annealing temperature (Table 1), and 30 s at 72 °C, followed by a final elongation step of 8–10 min at 72 °C. PCR products were analysed on CastAway 6% polyacrylamid 7 M urea precast gels (Stratagene Cloning Systems, La Jolla, CA, USA)