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Isolation and characterization of microsatellite loci in the Norway lobster (*Nephrops norvegicus*)

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Abstract

To assess the level and spatial pattern of genetic diversity of a marine crustacean species, *Nephrops norvegicus*, hypervariable molecular markers were obtained by screening of a partial genomic library enriched for microsatellite dinucleotide motifs. Following sequencing of the positive clones, 10 pairs of primers were designed and five of them were shown to detect polymorphic loci. These loci were checked for their variability in two natural populations, fished off the Portuguese coast. They showed a high variability for the number of alleles, and for the expected and observed heterozygosities. No significant differentiation between the two localities was observed.

Keywords: dinucleotide repeats, microsatellite, *Nephrops*

The Norway lobster, *Nephrops norvegicus*, is a crustacean species of high commercial interest in the Mediterranean and Atlantic. Some symptoms of overexploitation were detected via length frequency analysis (Sardá 1998) but a correct definition of management strategies for these populations requires the identification of the stock units as well as the potential for larval exchanges among them. Although no studies based on genetic markers were applied to confirm an overexploitation, a few studies have used allozymes to evaluate the geographical pattern of the genetic diversity of the Norway lobster, mainly in Mediterranean populations (Maltagliati *et al.* 1998). These biochemical markers revealed a low genetic diversity (mean heterozygosity) over the screened area. The low level of diversity observed for the allozymes on a large geographical scale could be due to biological traits or to the genetic marker itself. Hence, with the objective of a further fine geographical scale analysis, the development of more polymorphic markers is required. We present here the isolation and characterization of five polymorphic microsatellite loci in the Norway lobster.

Genomic DNA was isolated from fresh abdominal muscles following standard procedure (Sambrook *et al.* 1989) and digested with *Sau3A1* (Gibco-BRL, Life Technologies Inc., Gaithersburg, MD). The 300–800 bp fraction of the digested DNA was selected on agarose gel, purified and ligated into annealed *Sau3A* adaptors (AdapF: 5'-CTCTT-GCTTACGCGTGGACTC-3' and AdapR: 5'-GATCGAGTC-CACGCGTAAGCAAGAGCACA-3').

The enrichment procedure followed the protocol from Kijas *et al.* (1994) based on streptavidin-coated magnetic particles (MagneSphere, Promega, Madison, WI), with slight modifications. A 5'-biotinylated, 3'-aminated (CT)₁₅ oligonucleotide was used as a probe.

The enriched single stranded DNA was amplified using the primer adapF to recover double stranded DNA. The polymerase chain reaction (PCR) product was purified and ligated into the pGEM-T Easy vector (Promega, Madison, WI). The ligation was transformed into *Escherichia coli* cells (strain DH5 α). Approximately 500 white colonies were picked from agar plates into microplates containing 150 μ L LB/ampicillin solution and incubated overnight at 37 °C. Fifty μ L of each colony culture were transferred into new microplates and heated at 99 °C for 10 min for subsequent screening.

The screening for repeated motifs was inspired from Waldbieser (1995). Two PCR reactions were performed for each colony using the primers oligonucleotide (CT)₁₀, and M13 universal primer (Forward or Reverse for the two

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Table 1 Characterization of five microsatellite loci in *Nephrops norvegicus*. GenBank accession number, repeat units, primer sequences, annealing temperature (T_a) and magnesium chloride final concentration, *PCR product size deduced from the cloned allele, observed number of alleles (N_O), and numbers of individuals genotyped (n), observed (H_O), and expected (H_E) heterozygosity

Locus name	GenBank accession number	Repeat unit of cloned allele	Primers (5'-3')	T_a / MgCl ₂ (mM)	PCR product size*	N_O/n	H_O/H_E
Nnmic2-E4	AF221987	(TG) ₇ ... (GT) ₁₀	GCGTGTATGTCGATTTTACTG AGTAATGCCATCGCCGCTGTAGG	53/1.5	186	12/42	0.86/0.87
Nnmic1-F2	AF221988	(AAG) ₂₄ ... (AG) ₁₁	TGCTAAGCCTTCTGTCTGATAAGCTA ATTCTCGGAGGTAACCAGACTTTT	55/2	209	41/38	0.95/0.98
Nnmic1B11	AF221989	(GA) ₁₀	TACAACAAAGGGTCACTACAC CCATAGAGCTAACTAACGCAGG	48/1	149	12/38	0.84/0.76
Nnmic1-C12	AF221990	(GT) ₆	TGTCGTCGTAACTACCCGCTG ATACATCGTCCACTGCTCCAC	50/2	93	4/40	0.45/0.48
NnmicT-G2	AF221991	(CT) ₃₀	ACTGTCCGGTCAGGTTGAGA TCAGTCAAGAGATTGGGGAG	50/0.9	148	29/40	0.93/0.96

respective reactions). Plasmid DNA was prepared for the positive colonies, i.e. the ones showing a clear amplification product in one, or both reactions, and sequenced using the dideoxy chain termination method (Sanger *et al.* 1977). From the 60 sequences obtained, 10 pairs of primers were designed using the Primer Designer software (version 2.0, Scientific & Educational Software, 1990–91).

Microsatellite polymorphism was analysed by specific PCR for *c.* 40 individuals from two populations off the Portuguese coast (20 individuals from a west coast population and 20 from the south coast). The 10 μ L reaction mixture contained from 10 to 50 ng of template DNA, 1 μ M of each locus-specific primer (see Table 1), 60.225 μ M each dCTP, dGTP, and dTTP, 10 μ M of dATP, 0.08 μ L [α^{35} S]-dATP (12.5 mCi/mL, 1250 Ci/mmol), from 0.9 to 2 mM of MgCl₂ (Table 1), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, and 0.5 units of *Taq* DNA polymerase (GIBCO-BRL, Life Technologies Inc., Gaithersburg, MD). After a denaturing step of 4 min at 94 °C, samples were processed through 30 cycles consisting of 45 s at 94 °C, 45 s at T_a (see Table 1), and 45 s at 72 °C. A final elongation step was performed at 72 °C for 5 min. The reactions were performed in a Stratagene Robocycler (Stratagene Cloning Systems, La Jolla, CA). PCR products were denaturated and electrophoresed on standard DNA sequencing gels.

Allelic variation was analysed relative to two controls (i.e. two samples loaded every 10 lanes on each gel). If necessary, two or more runs were performed to verify the allele typing, by re-ordering the samples.

The five loci showed a high level of variability with a number of alleles from 4 to 41 per locus, and an expected heterozygosity H_E ranging from 0.48 to 0.98. The observed heterozygosities at the five loci were close to the expected values and no departure from the Hardy–Weinberg equilibrium

was observed within each population (GENEPOP 3.1b, Raymond & Rousset 1995). No global or single locus differentiation was detected between these two populations.

The high level of observed diversity at these five loci, therefore, seem suitable for further population analysis of *N. norvegicus* at a fine spatial scale.

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