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TECHNICAL NOTE

Development of twelve novel microsatellite loci in the European lobster (*Homarus gammarus*)

Carl André · Halvor Knutsen

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Abstract We developed twelve novel microsatellite loci primers in the European lobster (*Homarus gammarus*). All markers were obtained from partial genomic DNA libraries enriched for tetranucleotide repeats and characterized in 48 unrelated individuals from one putative population. The number of alleles ranged from 5 to 13, with an average of 8.3 per locus, and the observed heterozygosity ranged from 0.35 to 0.83 (average 0.69). These microsatellite loci can be used as markers in the assessment of connectivity and genetic structure of exploited lobster populations.

Keywords *Homarus gammarus* · Microsatellite primers · Polymorphism · Population structure

European lobster (*Homarus gammarus*) is a large decapod species of high importance in commercial and recreational fisheries from northern Norway to the Mediterranean Sea. Since the 1950s, European lobster has been in severe decline and is currently on historical low levels in Norway (Pettersen et al. 2009). While the general biology of European lobster is relatively well known, information about population structure relevant for management is scarce. Tagging studies indicate that adult lobsters are relatively stationary, although they may undertake migrations of several tens of km's (Smith et al. 2001). European lobster has a free-swimming larval stage that is planktonic for several weeks, and thus possesses a potential for long-range dispersal. However, little is known about the realized dispersal in natural lobster populations. Earlier genetic studies using allozymes, mtDNA and microsatellites indicate large-scale structure along the European coast (Jørstad and Farestveit 1999; Jørstad et al. 2004; Triantafyllidis et al. 2005). Recently, several studies have shown that many marine species are spatially structured into genetically distinct populations on remarkably fine geographic scales (e.g. Jorde et al. 2007). Knowledge about such small-scale population structure and connectivity relevant for the management of European lobster populations is presently lacking. Here, we present 12 microsatellite loci developed for *H. gammarus* suitable for the detection of potential population structure in this species.

We employed the company GIS (Genetic Identification Service Inc.) for the development of tetra repeat microsatellite loci. Methods for DNA library construction, enrichment and screening were as described previously (Jones et al. 2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, Bsr B1, *Pvu*II, *Stu*I, *Sca*I, Eco RV). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules. Libraries were prepared in parallel using Biotin-AAC(12), Biotin-CAG(10), Biotin-CATC(8) and Biotin-TAGA(8) as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hind*III to remove the adapters. The resulting fragments were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *E. coli* DH5α. Recombinant clones were selected at random for sequencing on an ABI 377, using ABI Prism Taq dye terminator cycle sequencing methodology.

C. André (✉)
Department of Marine Ecology-Tjärnö, University of Gothenburg, 452-96 Strömstad, Sweden
e-mail: carl.andre@marecol.gu.se

H. Knutsen
Flødevigen Marine Research Station, Institute of Marine Research, 4817 His, Norway

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Table 1 Primer sequences and characteristics of twelve microsatellite loci in the European lobster (*Homarus gammarus*)

Locus	GenBank acc no.	T_a (°C)	Repeat motif	Primer sequences (5′–3′)	Size range (bp)	N_A	H_E	H_O	F_{IS}	*P*-value
HGA8	XXXXXXXX	56	$(TATG)_{23}$ $(TTTG)_4$ $(TATG)_5$	F: TTGAACAGCAAAAACGTAGTG R: ACATCACACCACAACTCACTG	269–325	12	0.828	0.744	0.102	0.479
HGB4	XXXXXXXX	56	$(AAAC)_6$	F: TTCGCTAGTCCGTCTGTCC R: ACGAAGGATTACGGCACAT	187–231	6	0.676	0.574	0.151	0.162
HGB6	XXXXXXXX	56	$(CCAT)_{12}$	F: AGAAGGGAGGTGGGTGAG R: ATGAACCCGTCTGAGGTTATC	150–190	7	0.791	0.791	−0.000	0.325
HGC6	XXXXXXXX	56	$(TGTA)_{19}$	F: AGGCTGCATAGTTACACGTTTG R: ACCCAGTGTCAAGGAATAGTCC	274–318	6	0.383	0.354	0.076	0.228
HGC103	XXXXXXXX	56	$(GTAT)_{10}$	F: TGGTATTATGGCTACGACAAG R: CAAAAGACGGGTTTCAATC	220–254	5	0.686	0.744	−0.085	0.835
HGC111	XXXXXXXX	56	$(TAGA)_8$	F: TGAAGCGTGGAGGACCTT R: CACACCTGTCTGGCTACACC	258–280	10	0.828	0.787	0.050	0.077
HGC118	XXXXXXXX	56	$(TACA)_{10}$	F: TCGTTTCCAATGGTCTCG R: AAGTTGAAGGAGGTGCTTGAC	262–296	7	0.582	0.659	−0.133	0.457
HGC120	XXXXXXXX	56	$(GTAT)_9$	F: CCCTCTCTCATCCCTCTTATC R: ACCCTTATTCATCCATCCTTC	251–297	13	0.876	0.833	0.050	0.407
HGC129	XXXXXXXX	56	$(GTAT)_7$	F: TTGAACGCTATGAACTGAGAC R: AGGCATACAAATAAACGCAC	247–291	6	0.610	0.645	−0.058	0.911
HGC131b	XXXXXXXX	56	$(GTAT)_{21}$	F: CATGGGTGATTAGGATGACC R: TGGCACCATAGGTTCGTATC	226–276	12	0.843	0.808	0.042	0.214
HGD106	XXXXXXXX	56	$(CTAT)_9$	F: CATACCGAACCAAGTGTAAAC R: GCCCACAGTAACAGATAAGAG	139–167	7	0.685	0.760	−0.111	0.239
HGD111	XXXXXXXX	56	$(GATA)_8$	F: TAAAGGTGATGTTCAGTCCAC R: CTTGACCCGCTACCAATAC	231–275	8	0.619	0.586	0.053	0.571

Size range of fragments (bp), number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity and deviation from Hardy–Weinberg expectations (F_{IS}), are based on a sample of 48 individuals. Uncorrected *P*-values for two-sided tests

The optimal amplification reaction mix for all primer pairs consisted of 1× Biolase© Buffer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 6 M each primer (forward primer fluorescent-labelled), 0.025 U μl^{-1} Biolase© Taq polymerase, and 0.2 ng μl^{-1} template DNA in 50 µl final reaction volume. Samples were amplified in a Perkin-Elmer-Cetus thermal cycler by an initial three min of denaturation at 94°C, followed by 35 cycles of denaturation (94°C, 40 s), annealing (55°C, 40 s), and extension (72°C, 30 s), with final extension of 4 min at 72°C.

DNA from eight individuals collected in the Skagerrak Sea was extracted using the PureGene DNA Extraction Kit® kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturers instructions. Microsatellite loci were amplified in 10 µl reactions in the following reaction mix: $MgCl_2$, 2 mM; dNTPs (premixed), 0.2 mM each; primers, 0.3 µM each; BioTaq DNA Polymerase® (Bioline USA, Canton, MA, USA), 0.025 U μl^{-1}; template DNA, 0.2 ng μl^{-1}. PCR was conducted in a RoboCycler Gradient 96® thermocycler (Stratagene, Inc., La Jolla, CA, USA) by an initial denaturation (94°C, 3 min), followed by 35 cycles of denaturation (94°C, 40 s), annealing (55°C, 40 s), and extension (72°C, 30 s), and a final extension at 72°C for 4 min. PCR products were labelled using one of the conventional sequencing dyes NED, HEX or FAM (Applied Biosystems, Inc.). Amplification products were separated on polyacrylamide gels in an ABA 377 DNA sequencer and sized using Genotyper 2.5 software and Rox 400 HD size markers (Applied Biosystems, Inc., Foster City, CA USA). Four libraries were screened for the microsatellite motifs (AAAC)n, (CATC)n (TACA)n and (TAGA)n. A total of 100 clones were sequenced and 19 primer pairs designed using DesignerPCR, version 1.03 (Research Genetics, Inc.). These 19 primers were tested against 16 additional Skagerrak individuals resulting in twelve polymorphic and reliably amplifying loci.

Population screening of the twelve loci was conducted by analysing 48 individuals collected at Kåvra, Lysekil on the west coast of Sweden (58.33°N; 11.36°E). Genomic DNA was isolated using Viogene Blood and Tissue Genomic DNA Extraction Miniprep System (Viogene Inc.) according to manufacturer's protocol. PCR amplifications were carried out in 10 µl reaction volumes on Bio-Rad MYCycler, with fluorescently (CY-5) 5′-tagged forward primers (Sigma). The standard reaction composition included 1 µl of template DNA, corresponding to 20–40 ng, 10 × 15 mM $MgCl_2$ PCR buffer, 0.4 mM dNTPs, 0.125 mM of forward and reverse primer (Sigma) and 0.06 units μl^{-1} of Taq DNA polymerase (Qiagen. Inc.). Dilutions were done using Eppendorf Molecular Biology Grade Water. Thermal cycling conditions were as follows: An initial denaturation step at 94°C for 5 min, followed by 30 cycles of 95°C denaturation, annealing at 56°C (for all loci, see Table 1) and 72°C synthesis, each for 30 s. A final elongation step at 72°C for 15 min completed the amplification.

Allele sizes and genotypes were determined by fragment analysis using Beckman Coulter CEQ 8000 automated sequencer and included software (CEQ8000 Genetic Analysis System, version 8.0). We tested the loci for all individuals to assess gene diversity and evidence for linkage disequilibrium or deviation from Hardy–Weinberg expectations. Gene diversity and F_{IS} was estimated with GDA (Lewis and Zaykin 2001); significance of F_{IS} was assessed using the probability tests within GENEPOP on the web (http://wbiomed.curtin.edu.au/genepop/). The software MICROCHECKER (Van Oosterhout et al. 2004) was used to investigate the potential presence of null alleles or other technical artefacts. No locus deviated significantly from Hardy–Weinberg equilibrium (Table 1), or showed evidence of technical artefacts or null-alleles. Three out of 67 (4.4%) comparisons between pairs of loci displayed significant linkage disequilibrium (tested in GENEPOP), as expected from chance alone.

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