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Short Communication

Isolation and characterization of 13 polymorphic microsatellites for the black murex, *Hexaplex nigritus*

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ABSTRACT

Thirteen polymorphic microsatellite markers have been isolated and characterized for the black murex (*Hexaplex nigritus*). These loci are moderately to highly variable with seven to 37 alleles within 113 individuals from four populations in the Northern Gulf of California. Expected heterozygosities ranged from 0.43 to 0.98. High variability indicates that these markers will be useful for studying population structure and connectivity in this species.

1. Introduction

The black murex, *Hexaplex nigritus*, is a muricid gastropod endemic to the Gulf of California, Mexico (Cudney-Bueno and Rowell, 2008). The predatory snail forms aggregations in spring and summer during which females deposit egg capsules on the shells of conspecifics (Cudney-Bueno et al., 2008). These aggregations serve as habitat for a diverse community of juvenile invertebrates (Prescott and Cudney-Bueno, 2008). Commercial fishermen have exploited the aggregations or living reefs since the early 1990s resulting in declining stocks (Cudney-Bueno and Rowell, 2008). In a conservation effort and in order to address population structure and connectivity 13 polymorphic microsatellites loci were characterized (Table 1) and isolated from the black murex.

2. Materials and methods

DNA was extracted with DNeasy Blood and Tissue kit from Qiagen using approximately 20 mg of tissue. Genetic Identification Services (GIS, http://www.genetic-id-services.com) used 100 μg of genomic DNA from one individual to create genomic libraries, which were then enriched for CA-, CATC-, TACA-, and TAGA-microsatellites motifs. GIS identified 75 different microsatellite-containing clones and PCR primers were designed for 50 of those clones using DesignerPCR version 1.03 (Research Genetics, Inc.). We tested 45 loci for polymorphism. Amplification reactions were carried out in an Applied Biosystems GeneAmp PCR system 9700 using fluorescently labeled forward primers and cold reverse primers (Table 1) in a total of 10 μl containing 5 μl of 2× Qiagen Multiplex PCR mastermix (HotStarTaq DNA Polymerase, Qiagen Multiplex PCR buffer which contains 6 mM $MgCl_2$, pH 8.7 20 °C, and dNTP mix), 1 μl of 10× primer mix (containing each primer at 2 μM), 2.5 μl of RNase-free water, 1 μl Q-solution, and approximately 50 μg of DNA. The following temperature profile was used: 15 min at 95 °C to activate HotStarTaq DNA Polymerase, followed by 40 cycles of 30 s at 94 °C, 90 s at 56-58 °C, and 60 s at 72 °C and a final extension of 10 min at 72 °C.

Microsatellite amplification products were diluted with nano-water (1:5), mixed with Applied Biosystems GeneScan 500 Rox size standard marker, run on an ABI 3100 automated sequencer, and scored using GeneMapper version 3.7 (Applied Biosystems). We checked our data for null alleles using Micro-Checker (van Oosterhout et al., 2006) and used Arlequin version 3.11 (Excoffier et al., 2005) to calculate expected and observed heterozygosities and Fis values and to test for Hardy–Weinberg (HW) equilibrium and linkage disequilibrium (LD).

3. Results and discussion

Sixteen out of 45 loci reliably amplified products of the correct size. Two of these loci were determined to have null alleles for all of the populations by Micro-Checker (van Oosterhout et al., 2006). Another locus was determined to be out of HW in every population and exhibited LD. Table 1 summarizes the characteristics of the 13 polymorphic loci isolated for the black murex. These loci exhibited polymorphisms ranging from seven to 37 alleles per locus within 113 individuals from

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Table 1
Characterization of 13 polymorphic microsatellite loci for the black murex, *Hexaplex nigritus* from 113 individuals from four populations: 26 from Punta Chueca (PCH), 26 from El Borrascoso (EBO), 23 from Isla San Jorge (ISJ), and 33 from San Luis Gonzaga (SLG). Columns correspond to: microsatellite name (locus), forward (f) and reverse (r) primer sequences, repeat motif, annealing temperature in degrees centigrade (T_a), number of alleles per locus (N_a), amplification size of original clone (A_s), repeat size in base pairs (RR(bp)), observed (H_o) and expected heterozygosities(H_E) for each population.

							PCH		EBO		ISJ		SLG	
Locus	Primer sequence (5′-3′)	Repeat	T_a	A_s	N_a	RR(bp)	H_o	H_E	H_o	H_E	H_o	H_E	H_o	H_E
HNI_A3	F:CCATTGCTGAGAGACTGAAGAA R: ACATTTGCGCTTAGTTTGACTG	$(CA)_{22}$	58	250	15	238–268	0.72	0.83	0.77	0.83	0.85	0.84	0.91	0.84
HNI_A5	F: CTGTGCAACATCTCTCATTGTT R:ATTTTGCGCTATACCAAGAATG	$(TAA)_7$	57	170	7	164–182	0.45	0.46	0.39	0.43	0.41	0.55	0.36	0.39
HNI_A10	F:GAATCCATCCTATGTTTTCAAG R:AAAGAGAGAGGGGAAGAATAAG	$(CA)_{31}$	56	167	32	133–237	0.56	0.97	0.80	0.93	0.88	0.96	0.84	0.95
HNI_A12	F:AGTAGGCGGCATTTCACTTC R:CACGAAACTCTGCAAAGACG	$(CA)_{37}$	58	214	12	136–216	0.58	0.51	0.50	0.53	0.48	0.52	0.46	0.50
HNI_A108	F:TGAATAACTGGCTGTGTGTTC R:CCATTATCATCAGTTGACGC	$(CA)_{31}$	58	282	37	259–345	0.80	0.96	0.81	0.98	0.92	0.97	1.0	0.98
HNI_A117	F:GGCAGAACGGCATTAACTATG R:CAGGGATCGACAGAGAATCAG	$(TCTG)_8$	57	136	7	120–138	0.46	0.43	0.50	0.50	0.32	0.46	0.36	0.49
HNI_A120	F:CTAGCCCCAGTGTATGGTC R:GGTGTCAGTCCTCATTTGG	$(CA)_{21}$	57	220	30	202–282	0.79	0.93	0.83	0.95	1.0	0.97	0.81	0.95
HNI_B9	F:GGGGTCTACAACACGGTG R:GATGGGAATGGATGGTTG	$(CATC)_{19}$	56	133	10	121–161	0.48	0.68	0.62	0.61	0.61	0.64	0.64	0.61
HNI_B12	F:CACGCACACGTTATACATACAC R:CTTATTCTTCCCCTCTCTCTTT	$(CA)_{51}$	58	287	22	267–329	0.16	0.56	0.21	0.57	0.36	0.82	0.68	0.72
HNI_B104	F:ATCGAAGAAGTGGGCATATTG R:ACTGGTAAGATGGGGTTGTTG	$(CATC)_{14}$	57	177	23	153–215	0.88	0.86	0.62	0.85	0.89	0.91	0.91	0.91
HNI_B120	F:GCAAACACACTCACACACTTT R:CATCCAAGTAAGCAGGAAGAC	$(CTAC)_{26}$	57	280	22	240–286	0.69	0.83	0.75	0.89	0.73	0.80	0.59	0.83
HNI_C12	F:TGTCGAATACGATGGAGAGTG R:GGTCTGCTTTACCATTGGAAG	$(TACA)_{23}$	58	232	16	229–301	0.73	0.78	0.62	0.83	0.67	0.84	0.66	0.85
HNI_C102	F:TGAGGCTTCGTGTTGAAG R:CGTCATAAATGCAAACATAGTG	$(TACA)_{21}$	57	163	19	109–189	0.60	0.84	0.80	0.81	0.71	0.79	0.88	0.88

four populations: Punta Chueca, El Borrascoso, Isla San Jorge, and San Luis Gonzaga. Expected heterozygosities ranged from 0.43 to 0.98 and Fis values ranged from −0.13122 to 0.72379. Each population had one to three loci out of HW equilibrium but no locus was out of HW equilibrium in every population. Table 2 displays the Fis values and the results for HW equilibrium tests for all loci in each population. Arlequin (Excoffier et al., 2005) detected LD in multiple loci pairs in each population but only one pair, A108 and A120, was in LD in every population. No more than two peaks were detected at any locus while scoring with GeneMapper. High variability indicates that these markers will be useful for studying population structure and connectivity of this commercially and ecologically important species.

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Table 2
Inbreeding coefficient (Fis) values and Hardy–Weinberg Equilibrium test results for all loci in four populations; Punta Chueca (PCH), El Borrascoso (EBO), Isla San Jorge (ISJ), and San Luis Gonzaga (SLG). (*Indicates the locus in that population is out of HW equilibrium).

Loci	PCH	EBO	ISJ	SLG
HNI_A3	0.13166*	0.07236	−0.07923	−0.00640
HNI_A5	0.01176	0.09584	0.07134*	0.26761
HNI_A10	0.42515*	0.14132*	0.11709	0.08808
HNI_A12	−0.13122	0.06205	0.09263	0.08277
HNI_A108	0.16739*	0.17375*	−0.02590	0.04913
HNI_A117	−0.07914	0.00154	0.25581*	0.29870
HNI_A120	0.15310	0.12879	0.15302	−0.03616*
HNI_B9	0.29842	−0.00629	−0.04429	0.05263
HNI_B12	0.72379*	0.64174*	0.05334*	0.56364
HNI_B104	−0.01931	0.27733*	0.00000	0.02194
HNI_B120	0.16512*	0.16109	0.28779	0.09351*
HNI_C12	0.06537	0.26403*	0.23276*	0.21013*
HNI_C102	0.28994*	0.00621	0.00230*	0.10224

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