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Research Note

SSRs in *Octopus mimus*: development and characterization of nine microsatellite loci

SSRs en *Octopus mimus*: desarrollo y caracterización de nueve loci microsatélites

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Abstract.- Nine microsatellite loci were developed for *Octopus mimus*, a cephalopod of commercial importance for artisanal fishermen. Genetic variation at these loci was examined in samples from Clavelito, a Benthic Resources Management Area (AMERB, in Spanish). All nine loci were highly polymorphic, with the number of alleles per locus ranging from 4 to 28 and the expected heterozygosity ranging from 0.651 to 0.946. These markers will be useful to address issues of population genetics, ecology, conservation and fisheries management related to that species.

Key words: Population genetics, AMERBs, cephalopod

Introduction

To evaluate how genetic variability is distributed it is necessary to use polymorphic molecular markers. In the last few years, microsatellites have been some of the most used molecular markers for determining genetic variability and genetic structure levels (Barbará *et al.* 2008). These codominant markers are higly polimorphic, with motifs of 1 to 6 nucleotides in length, organized in tandems, widely distributed along the genome (Tautz 1989).

In cephalopods as in other marine groups, few studies have been devoted to identify and characterize polymorphic microsatellite loci. This is unusual, due to the low genetic diversity that has been observed using others molecular markers in cephalopods (*i.e.,* Allozymes: Brierley *et al.* 1995, Triantafillos *et al.* 2004 and mitochondrial DNA: Oosthuizen *et al.* 2004).

Octopus mimus (Gould, 1852), is a member of Octopodidae Family, it inhabits rocky shore systems in the Southeast Pacific from northern Perú to central Chile. Biological traits such as separate sexes, internal fertilization and a semelparous reproductive strategy (Leite *et al.* 2008) make this species a good model to answer questions about genetic connectivity. Most studies in the Genus *Octopus* have focused on determining the phylogeny (*e.g.*, Warnke 2004) and only a few focus on their population biology. Even a smaller number of these studies used microsatellite loci as a tool for determining genetic structure *(e.g.*, Greatorex *et al.* 2000, Cabranes *et al.* 2008, Doubleday *et al.* 2009). Up until now, there is no information about *O. mimus* population genetics. In this paper, we describe the isolation, characterization and utility of nine microsatellite loci for *O. mimus* in one Chilean resource management area.

Materials and methods

High molecular weight DNA was extracted from arm tissue of 20 individuals from both sexes using a salting-out protocol (Jowett 1986). The genomic library was developed by Genetic Identification Services[1]. Briefly, the DNA was partially digested with a cocktail of seven blunt-end restriction enzymes (*Hae*III, *Stu*I, *Eco*RV, *Sca*I, *Bsr*BI, *Pvu*II, *Hiu*CII). Fragments between 350 and 700 bp were selected by gel extraction and ligated to a 20 bp oligonucleotide adaptor containing a Hind III restriction site at the 5' end. Microsatellite enrichment was achieved using streptavidin-coated magnetic beads and 5'-biotinylated CA_{15}, AAC_{12}, $TACA_8$ and $TAGA_8$ oligonucleotide probes. The captured molecules were amplified by PCR using a primer complementary to the adaptor, digested with *Hind*III to remove the adaptor, and ligated into the *Hind*III site of the pUC19 vector. The plasmids were then electroporated into *Escherichia coli* DH5. Recombinant clones, identified by blue-white

[1]Genetic Identification Services, Chatsworth. <http://www.genetic-id-services.com>

selection, were chosen arbitrarily for sequencing on an ABI 377 using the Big Dye Terminator Cycle Sequencing methodology (Applied Biosystems). Specific primers flanking the identified microsatellite sequences were designed using Designer PCR version 1.03 (Research Genetics) (Table 1). The microsatellite loci were amplified in 10 µl reactions containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM forward primer (fluorescently labeled), 0.2 mM reverse primer, 0.2 mM dNTPs, 0.1 U μl^{-1} Taq DNA polymerase (Invitrogen), and 20 ng of genomic DNA template. PCR was performed in a PTC-200 (MJ Research) thermal cycler with the following parameters: 94ºC for 3 min, followed by 34 cycles of 94ºC for 40 s, 56ºC for 40 s, 72ºC for 30 s, and a final extension at 72ºC for 4 min. All loci successfully amplified under the same conditions. The PCR products were analyzed on an ABI 3330 DNA sequencer. Alleles were scored using Peak Scanner v1.0, with GS500 (Applied Biosystems) as the internal size standard.

To characterize the polymorphism of each locus we used 100 individuals from Clavelito, Chile. To evaluate the potential presence of null alleles and genotyping artifacts (stutter bands or large dropout alleles) we first checked the data set with MICRO-CHEKER v2.2.3 software (Van Oosterhout *et al*. 2004). The number of alleles (Na), the expected (He) and observed (Ho) levels of heterozygosity were obtained using GENALEX v6 (Peakall & Smouse 2006). Deviations from Hardy-Weinberg equilibrium (H&W) and gametic disequilibrium between markers were tested using ARLEQUIN v3.1 (Excoffier *et al.* 2005). All probability values were estimated using 10000 permutations.

Results and discussion

We observed a significant departure from HWE in four loci (*i.e., Omim*A2, *Omim*B111, *Omim*C1 and *Omim*C122). None of the studied loci showed gametic disequilibrium. On the other hand, the results did not show any score errors like large allelle dropout and stutter bands. Two loci showed heterozygotes deficiency: *Omim*A105 and *OmimB*111 (Table 1) which, in this case, could be attributed mainly to: a) admixture of individuals captured over a large geographical area that may include more than one panmictic unit (Wahlund effect), b) null alleles, as some of the sequences (allele) could not be amplified, the number of heterozygotes is sub estimated, c) imbreeding

Table 1. Characteristics of the microsatellite loci isolated for the *Octopus mimus*. The values reported for each marker are the allele size range (bp), the number of alleles (Na), the observed (Ho) and the expected (He) heterozygosity, and the GenBank accession number. *Omim*: *Octopus mimus*. *: *P* < 0.05; **: *P* < 0.01 / Características de los loci microsatélites aislados en *Octopus mimus*. Los valores reportados para cada marcador son el rango del tamaño alélico (pb), el número de alelos (Na), la heterocigosidad observada (Ho) y esperada (He), y el número de acceso a GenBank. *Omim*: *Octopus mimus*. *: *P* < 0,05; **: *P* < 0,01

Locus	Nº Genbank	Primer sequence (5'-3')	Repeat unit	Size range (bp)	Clavelito		
					Na	Ho	He
*Omim*A2	GU584076	F: TCAATGGATGTTGATGTCAGA G R: TTCCAGGCAAGGTAATATCTTG	$(AT)_4(GT)_3N_2(GT)_6N_4(GT)_3N_7(AT)_5N_2(GT)_{14}$	268-306	8	0.893	0.816*
*Omim*A103	GU584077	F: CATTGCTGAACGAAGAACTC R: GCTTATCCCGAACAGGTAAT	$(ATCT)_6$	218-350	28	1	0.939
*Omim*A104	GU584078	F: TGGTTGATAGAAACTGTGTCG R: GTTGTGGTGATGGTGACTG	$(CCA)_6N_3(CCA)_2$	186-276	13	0.936	0.880
*Omim*A105	GU584079	F: GCCCTTTTGTTGTAATAATACG R: TTCTCGCTAATGGCAGTTC	$(AC)_{28}$	194-270	24	0.850	0.946
*Omim*B101	GU584080	F: CACCAAGTCACAGACATCTTG R: GCCAGATAAACAACAATAGCTG	$(CA)_{54}$	206-256	4	0.467	0.651
*Omim*B111	GU584081	F: TCCTGATTCAGTCATTTACAGG R: GGGTTCTGTTTCTTTGACATG	$(GT)_5(GA)_{15}$	186-300	8	0.273	0.826**
*Omim*C1	GU584082	F: CGTGTGTCAACCAATTTC R: GGTGTAGTTAAATGGCATACTC	$(ATGT)_{13}(GT)_{12}N_2(GT)_5N_2(GT)$	133-213	14	0.938	0.805**
*Omim*C106	GU584083	F: TGCCATCAAAGTGACACTG R: AACCACAGATACTCGAAGAATG	$(TGTA)_{20}N_4(TGTA)_9$	188-300	10	0.862	0.747
*Omim*C122	GU584084	F: ATTGTTGAAATCGCAACTGT R: AATTCTCATAATCGCTGTTGTG	$(ACT)_{19}$	118-256	17	0.942	0.800**

and d) selection. According to our results, null alleles are the most plausible cause of heterozygotes deficiency at these two loci, as suggested by the excess of homozygotes obtained by the Micro-Checker software. Heterozygosity deficits due to null alleles have been recorded in *Octopus* species, (*e.g.*, *O. vulgaris*, Casu *et al.* 2002, Cabranes *et al.* 2008, *O. maorum*, Doubleday *et al.* 2008, *O. maya*, Juárez *et al.* 2010). The presence of heterozygosity deficit could support the low variability recorded in other molecular markers used in cephalopods (Allozyme, Maltagliati *et al.* 2002; mitochondrial DNA, Oosthuizen *et al.* 2004). Moreover, life history traits like semelparity, low fecundity and territorial behavior could promote inbreeding and consequently the heterozygosity deficit.

The Na per locus ranged from 4 to 28, the Ho from 0.273 to 1 and the He varied from 0.651 to 0.946 (Table 1). Polymorphism found in microsatellite loci of *O. mimus* is comparable with other studies in other members of the genus *Octopus*. He in *O. mimus* (mean He = 0.823) was less than He in *O. vulgaris* (mean He = 0.874, Cabranes *et al.* 2008; mean He = 0.91, Murphy *et al.* 2002) and *O. maorum* (mean He = 0.85, Doubleday *et al.* 2008). On the other hand, average He in *O. mimus* was greater than He in *O. maya* (mean 0.645, Juárez *et al.* 2010) and *O. vulgaris* (mean He = 0.765, Greatorex *et al.* 2000).

In summary, this work provides a significant genetic tool for future population genetic studies, which should improve government management policies to maintain the sustainability of these important fisheries, and helping not only to promote the conservation of the fishing stock, but also to conserve genetic diversity of this cephalopod along the Chilean coast.

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