ONLINE RESOURCES

Isolation and characterization of microsatellites for jumbo squid *Dosidicus gigas* (Ommastrephidae)

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Introduction

The jumbo squid, *Dosidicus gigas* (D'Orbigny 1835) is one of the largest and most abundant members of the family Ommastrephidae. The highest concentrations of *D. gigas* are found in Peru, Chile and the Gulf of California (Mexico), where it is an important fishery resource. Current knowledge of the species suggests a strong spatial and temporal variability in population structure, mainly by size and maturity, both within the Gulf of California (Markaida and Sosa-Nishizaki 2001; Morales-Bojórquez *et al*. 2001) and in other geographical regions (Tafur *et al*. 2001). *D. gigas* exhibits 'offshore–onshore' migrations up and down the shelf slope. In addition, the spawning aggregations for *D. gigas* are geographically widespread with different maturation and growth rate and thereby may promote intraspecific genetic variation within stocks (Anderson and Rodhouse 2001). It is an endemic species from the Eastern Pacific, ranging from California, USA (30°N) in the north to central Chile (20°S) in the south (Nesis 1983). Recently, a range extension was recorded as far north as Canada (Cosgrove 2005; Field *et al*. 2007; Zeidberg and Robinson 2007).

The use of morphometric and/or meristic characters in this group is problematic due to plasticity of body form and growth (Shaw 2002). Three different groups of *D. gigas* have been recognized based on mantle length (ML) at maturity. A small size at maturity form comprises animals of 13–14 cm ML and is thought to be limited to equatorial waters. A medium size at maturity form, maturing at 24–28 cm ML, occurs across the entire species range, and the northern and southern peripheries of the species range are inhabited by a large size at maturity form that matures at 40–55 cm ML or greater (Nigmatullin *et al*. 2001).

The aim of this study was to isolate and characterize microsatellite markers in *D. gigas* for later use in population genetic studies. We describe nine polymorphic microsatellites, although most of them have a deficit of heterozygotes apparently caused by a high frequency of null alleles.

Materials and methods

Genomic DNA was obtained from the arm muscles of *D. gigas*, previously preserved in ethanol, using a standard phenol–chloroform extraction protocol (Sambrook *et al.* 1989). A microsatellite-enriched genomic library was constructed following a protocol modified from Glenn and Schable (2005). Five micrograms of DNA from 20 pooled individuals were digested with *Rsa*I and *Sau*3AI, the fragments were ligated to double-stranded SuperSNX-24 linkers. To ensure ligation was successful, linker-ligated fragments were amplified using the polymerase chain reaction (PCR) and SuperSNX-24 forward primer. The library was screened with biotinylated dinucleotide and tetranucleotide microsatellite probes (GT_{15}, CT_{15}, $GATA_{10}$ and $CTGT_{10}$). Hybridized fragments were captured on streptavidin-coated paramagnetic beads (Dynal), recovered again by PCR reactions. The PCR products were ligated and transformed using a TOPO TA Cloning kit (Invitrogen, Grand Island, USA). Two-hundred positive colonies were amplified with M13 forward and reverse primers, and 113 clones ranging in size from 300–1200 base pairs (bp) were selected for sequencing. Fifty-five clones (48.6%) contained microsatellite sequences, of which 25 proved to be suitable for primer design. Eighteen primer pairs were designed using Primer3 software (Rozen and Skaletsky 2000) of which only nine loci amplified using a standard protocol and were scored on 27 individuals from the Gulf of California, Mexico (table 1). Remaining loci

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Table 1. Characteristics of nine microsatellite loci in jumbo squid *D. gigas*.

Locus (pb)	Repeat motif	Sequence (5′–3′)	T_a (°C)	*N*	Size range (pb)
DGI10	$(GTCT)_6(TCGT)_{10}$	F: CATCCTTCTATCTGTTGTCTA R: AGTTAAGAAATGAAAGTGAGTC	58	26	248–300
DGI68	$(CT)_7$	F: GACACATTTGATCCATTGGTAA R: GCAGCGTTAGGTAAGTGCAA	58	27	192–286
DGI85	$(GAT)_{20}$	F: ATGGAAGGAGTTGGGAAAGG R: GATAAGCGGTTCATCATTCAAA	56	26	192–262
DGI90	$(AC)_7$	F: TTCATGCTCATTCCCTTTCAC R: TCTGTGCGAATATGCATTCTG	56	26	110–160
DGI11	$(TC)_{28}(AC)_7$	F: TTCATGGCTTACATCTTGCTG R:GTCGTAGAGGCGAAGAAACG	57	27	152–260
DGI61	$(TATC)_{20}$	F: AATCGATTCTATTCTGTTCAGC R: AGAGGAAATTTGATCTCGATTG	58	23	154–256
DGI71	$(TG)_7$	F: CAGAATGGGCACGTATGC R: TTCCCTTTCACTCACTTGCTC	56	24	130–170
DGI66	$(AC)_8$	F: CATGGACCGATCCCTTAATTC R: GATCATTGAGCATGCAGGTG	56	22	133–165
DGI8	$(GA)_{12}\ldots(GA)_7$	F: GCCATATGTGAAAGACATGGTG R: CTAGCAACTGTTCGCCAACTC	57	26	182–258

Primer sequences (F, forward; R, reverse); T_a, annealing temperature; *N*, sample size.

presented stutter bands or either the flanking regions were too short and failed to meet minimum amplification criteria.

Amplifications for microsatellite loci were performed in 15 μL volume containing ~50 ng genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM each dNTPs, 0.2 μM each primer, 0.2 U *Taq* polymerase and 1.5 μL of the manufacturer's supplied 1× PCR buffer (Invitrogen). Cycling conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, locus-specific annealing temperature (table 1) for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR was carried out in iCycler thermal cycler (Bio Rad, USA).

Amplified products were resolved at 6% polyacrylamide gels 7.5% urea, revealed with SYBR®Gold nucleic acid stain (Invitrogen) and visualized in the scanner FMBIO III Multi Viex (Hitashi, USA). Allele sizes were determined using size standards along with a 10-bp DNA ladder (Invitrogen). Observed and expected heterozygosity were calculated with GenAlEx 6.3 (Peakall and Smouse 2006) and deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were estimated with GenePop 4.0 (Rousset 2008). MicroChecker 2.2.3 (Van Oosterhout *et al*. 2004) was used to check for null alelles.

Results and discussion

Nine highly polymorphic microsatellite markers were developed between 4 and 22 alleles. Expected and observed heterozygosity ranged from 0.763 to 0.961 and from 0.333 to 0.827, respectively (table 2). No significant LD was found between any pair of polymorphic microsatellite, but loci *DGI68, DGI11, DGI61* and *DGI71* deviated significantly from HWE due to heterozygote deficiency. According to the expected distribution of homozygote size classes calculated with Micro-Checker 2.2.3 (Van Oosterhout *et al*. 2004), the

Table 2. Genetic diversity of nine microsatellite of *D. gigas*.

Locus	*N*	N_A	H_E	H_O	*P* value	GenBank accession no.
DGI10	26	12	0.853	0.827	0.456	KF053137
DGI68	27	17	0.959	0.813	0.000*	KF053138
DGI85	26	17	0.924	0.825	0.074	KF053139
DGI90	26	16	0.961	0.691	0.212	KF053140
DGI11	27	22	0.947	0.753	0.000*	KF053141
DGI61	23	10	0.917	0.333	0.000*	KF053142
DGI71	24	10	0.842	0.333	0.000*	KF053143
DGI66	22	4	0.763	0.600	0.083	KF053144
DGI8	26	16	0.786	0.654	0.134	KF053145

N, sample size; N_A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.
* Significant deviation from HWE.

presence of null alleles was suggested for those loci ($P < 0.01$). Null alleles have been observed previously in a wide variety of taxa at frequencies from 20 to 40% (Dakin and Avise 2004), and at even higher frequencies in some mollusc and insect species (Chapuis and Estoup 2007).

The relatively low success rate of the primers developed here may due in part to high intraspecific level of genomic polymorphism; Reece *et al.* (2004) attributed variation in amplification efficiency and the occurrence of null alleles in *Crassostrea virginica* (eastern oyster) to polymorphisms in the microsatellite-flanking regions. Further, previous studies in gastropods suggested that genomic complexities such as cryptic repetitive DNA and sequence similarities in flanking regions might hamper the development of PCR primer sets for amplification (McInerney *et al.* 2011). Despite a widespread utility of microsatellite markers in cephalopods, several studies have reported presence of non amplifying alleles resulting from mutations in primer sites (Perez-Losada *et al.* 2002; Naud and Shaw 2008; Iwata *et al.* 2008). Redesign of primers and rescreening from individuals will be required to confirm that variation in the microsatellite-flanking regions was responsible for the varied success in amplification. Nonetheless, based on our data at least five of the loci developed here will be useful for the study of population genetics in *D. gigas*.

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