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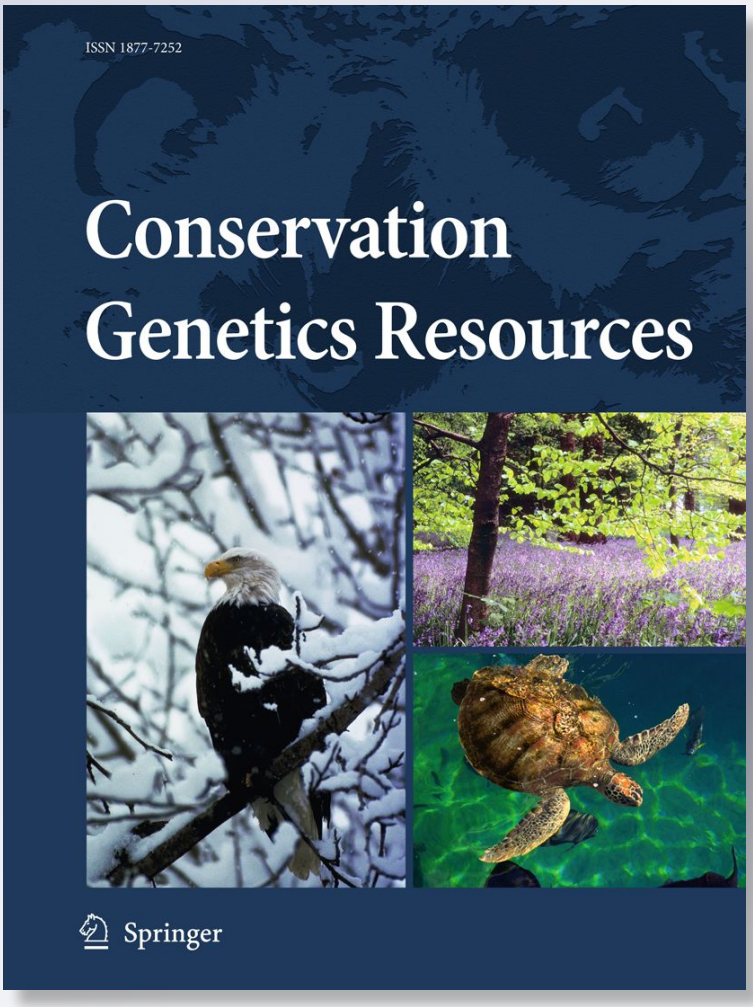

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TECHNICAL NOTE

Microsatellite marker isolation and development for the giant Pacific Octopus (*Enteroctopus dofleini*)

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Abstract We isolated and developed 18 novel microsatellite markers for the giant Pacific octopus (*Enteroctopus dofleini*) and examined them for 31 individuals from Prince William Sound (PWS), Alaska. These loci displayed moderate levels of allelic diversity (averaging 11 alleles per locus) and heterozygosity (averaging 65%). Seven loci deviated from Hardy–Weinberg Equilibrium (HWE) due to heterozygote deficiency for the PWS population, although deviations were not observed for all these loci in other populations, suggesting the PWS population is not in mutation-drift equilibrium. These novel microsatellite loci yielded sufficient genetic diversity for potential use in population genetics, individual identification, and parentage studies.

Keywords Microsatellite markers · *Enteroctopus dofleini* · Population genetics

The giant Pacific octopus, *Enteroctopus dofleini* (Wülker 1910), previously *Octopus dofleini* (Hochberg 1998), is the world's largest octopus species. This species occurs from the intertidal (Scheel 2002) to depths of possibly 1,000 m (Hochberg 1998) and ranges from California northwest along the Alaska coast and west to Japan. The species has a complex taxonomic history, due to systematic changes used for cephalopod identification (Nixon 1998), and to variable morphological characteristics reported across its range (Pickford 1964; Hochberg 1998). Species complexes have been identified or suggested based on geographic ranges and key morphological characteristics (Berry 1912; Pickford 1964) however; Hochberg (1998) did not support these and suggested the existence of a species complex.

Enteroctopus dofleini are caught as a major component of by-catch in pot, long-line, and trawl fisheries (Reuter et al. 2010) throughout Alaskan waters. A proposed amendment to the Fisheries Management Plan for Groundfish in the Gulf of Alaska would require population data for octopuses, currently lacking, to meet management requirements (Reuter et al. 2010), thus creating a need for genetically based population data.

Barry (2010) used mitochondrial DNA to examine *E. dofleini* population structure in Alaska. His results suggested the possibility of a cryptic species, and recommended further analyses using nuclear markers, including microsatellites. Microsatellite markers are still considered the marker of choice for population level studies, individual identification, and parentage studies (Buford and Wayne 1993; Sunnucks 2000).

Whole genomic DNA was extracted from tissue samples ($n = 31$) collected from giant Pacific octopus occupying intertidal and deep waters (>50 m) in Prince William Sound (PWS), Alaska, and isolated using a chloroform-isoamyl CTAB-PVP extraction technique modified after Stewart and Via (1993). Microsatellite motifs and flanking regions were isolated and sequenced by Genetic Identification Services (Chatsworth, CA) from one of these tissue samples. We designed primers from these sequences. The forward primer for each locus was synthesized with a modified, universal tail (M13F, M13R, or SP6; Table 1) added to the 5′-end (Oetting et al. 1995).

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Table 1 Characterization of 18 novel microsatellite loci and primer sequences developed for the giant Pacific octopus (*Enteroctopus dofleini*) surveyed in samples ($n = 31$) from Prince William Sound, Alaska

Locus name	Repeat motif	Primer sequence (bp)	Size range	GenBank accession number
Edoμ4	$(CA)_{12}$	F: M13F-AGCCCTCGAGCATATTCG	148–168	JQ236863
		R: TGATGTGGTTCCAGTGTC		
Edoμ6	$(CA)_4G(CA)_{23}(TA)_2CATA(CA)_9$ $(TG)(CA)_5(TA)(CA)_2$	F: M13F-TTAGTTGGATTGCTGGGC	180–220	JQ236864
		R: CAGTGGATCAACAATAGC		
Edoμ8	$(CA)_2(GA)(CA)_2CGACAAATA$ $(CA)_{27}AG(CA)_5AG(CA)_3$	F: M13F-GCCAATCACAAATAGTGAC	179–233	JQ236865
		R: CTACTTAGGTGTACGTGC		
Edoμ103	$(CA)_{13}$	F: SP6-TTTCTGAAGCGAGATCCG	144–156	JQ236866
		R: GTATCGTGAGTGTGTGC		
Edoμ104	$(CA)_8$	F: M13R-TCAGCAGACACATAGTGG	117–123	JQ236867
		R: GTTTGATCTGACTGTGTTG		
Edoμ105	$(CA)_{11}CN(CA)_9$	F: M13F-ACTCTCTCTTGCACATGC	187–205	JQ236868
		R: CCCTTGATTAGACAGATG		
Edoμ106	$(CA)_8TA(CA)_5$	F: M13R-CTGGGTTCATTCTCTTGG	110–126	JQ236869
		R: TACMCCAATGAGATATGC		
Edoμ109	$(CA)_7AA(CA)_{28}(TA)_2CAGA(CA)_3CG(CA)_5$	F: SP6-TGTTGCCCCACTGGTACG	179–193	JQ236870
		R: GAATGAGTCCATAATGGC		
Edoμ110	$(CA)_{12}$	F: SP6-TGAATCACCTACCCATCC	124–170	JQ236872
		R: GCTACATACACTCACACA		
Edoμ115	$(CA)_{18}$	F: M13R-CAGGGAAACCGAACTTGC	172–186	JQ236871
		R: ATACGTACAACTGCTGGC		
Edoμ118	$(CA)_{28}$	F: SP6-GGCGACAGTGATCATAGC	225–269	JQ236873
		R: TTTTCATACCCATCCAGC		
Edoμ122	$(GT)_{38}$	F: M13F-GGATGTTTCTGAGTAGCAGC	178–236	JQ236874
		R: TGTAGCTCAAGTAAGACC		
Edoμ123	$(CA)_{15}$	F: M13R-GATATGCAACATCTCAGC	167–177	JQ236880
		R: CAGTACGGAGTTAGTGGC		
Edoμ124	$(CA)_{26}$	F: M13R-GAATGTATGTACCAACGG	214–232	JQ236875
		R: CAACTTCTGGGATTTAGC		
Edoμ125	$(GT)_3CT(GT)_{27}$	F: SP6-TTGGAACACTCATTATCG	158–268	JQ236876
		R: GGTGAAAGGCAAAGTCGC		
Edoμ129	$(CA)_{17}$	F: SP6-TTAATGACCTGTTCACTG	127–159	JQ236877
		R: TGCATGTCTAACGTGAGG		
Edoμ216	$(CA)_{10}(TA)_4$	F: M13R-TACGATTGTAAATCCGGTAGC	113–125	JQ236878
		R: GTATAATCACCATCATCG		
Edoμ11	$(GA)_{13}AA(GA)_2AA(GA)_{23}$	F: M13R-GCATGATTTAACACTATGC	151–219	JQ236879
		R: ACAGTTTATTTCCCCTGC		

DNA fragments containing microsatellite were amplified using polymerase chain reaction (PCR). PCR amplifications were conducted in 10μL reaction volumes containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris/HCl, 0.1% gelatin, 0.2 mM dNTP's, 0.001 mg BSA, 0.3 units Taq polymerase, 0.5 pmol of each unlabeled primer, 1.5 pmol of labeled primer, and 1μL of 50 ng/μL of template DNA. Six loci (*Edoμ*6, 106, 110, 118, 122, and 125) also contained 500 mM of Betaine. The thermalcycler profile consisted of a 2 min denaturation at 94°C, followed by 40 cycles of 15 or 30 s at 94°C, 15 or 30 s at 50°C, and 30 or 60 s at 72°C. A final extension of 30 min at 72°C was performed. PCR-amplified product was resolved by electrophoresis on a 25 cm, 6% polyacrylamide gel and genotyped using a LI-COR (4200LR) automated DNA sequencer. Allele size for each locus was assigned via comparisons with a fluorescently labeled M13 sequence of known size (Amersham Pharmacia Biotech, Piscataway,

Table 2 Genetic diversity of 18 polymorphic microsatellite loci developed for *Enteroctopus dofleini* based on samples ($n = 31$) collected from Prince William Sound, Alaska

Locus	Number of alleles (A)	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Polymorphism information content (PIC)	Parental exclusion probability (Q)	Probability of identity (P_{ID})	Probability of identity (first order) (P_{IDsib})
Edoμ4	9	**0.448**	**0.819**	0.78	0.146	0.0460	0.362
Edoμ6	13	**0.480**	**0.808**	0.77	0.171	0.0403	0.369
Edoμ8	15	0.867	0.854	0.83	0.728	0.0229	0.340
Edoμ103	6	0.483	0.540	0.48	0.173	0.2450	0.553
Edoμ104	4	**0.433**	**0.543**	0.47	0.136	0.2600	0.554
Edoμ105	5	0.333	0.326	0.30	0.078	0.4450	0.709
Edoμ106	2	0.069	0.131	0.12	0.004	0.7500	0.878
Edoμ109	7	0.793	0.698	0.64	0.586	0.1250	0.444
Edoμ110	15	**0.774**	**0.897**	0.88	0.570	0.7110	0.860
Edoμ115	7	0.600	0.659	0.62	0.291	0.1280	0.465
Edoμ118	18	0.897	0.926	0.90	0.788	0.0060	0.299
Edoμ122	27	**0.821**	**0.939**	0.92	0.639	0.0030	0.292
Edoμ123	3	0.483	0.482	0.39	0.173	0.3430	0.604
Edoμ124	9	0.750	0.718	0.66	0.510	0.1120	0.431
Edoμ125	28	**0.759**	**0.968**	0.95	0.525	0.0004	0.276
Edoμ129	10	0.774	0.775	0.73	0.552	0.0640	0.390
Edoμ216	6	**0.290**	**0.613**	0.57	0.060	0.1560	0.495
Edoμ11	16	0.933	0.914	0.89	0.864	0.0090	0.305

Values in bold signify deviations from HWE ($P < 0.05$)

NJ). For quality control purposes, DNA was re-extracted, re-amplified, and re-analyzed for a minimum of 10% of samples for each locus.

We tested for significant deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium using GENEPOP 3.2 (Raymond and Rousset 1995). We used Microsatellite Toolkit (Park 2001) to determine number of alleles (A) and estimated observed (H_O) and expected (H_E) heterozygosity; PowerStat ver. 1.2 (Promega Corporation 2011) to generate polymorphism information content values (PIC; Botstein et al. 1980) and parental exclusion probability (Q; Weir 1996); and GIMLET (Valieré 2003) to estimate probability of identity values (P_{ID} and P_{IDsib}; Waits et al. 2001).

The number of alleles at each locus varied from 2 to 28 (mean = 11 per locus); H_O ranged from 0.069 to 0.93 (mean = 0.61); and H_E ranged from 0.13 to 0.97 (mean = 0.70) (Table 2). Seven loci deviated from HWE expectations ($P < 0.05$) due to heterozygote deficiency (Table 2). We found no evidence of linkage disequilibrium based on 153 pairwise comparisons ($P < 0.05$, Bonferroni correction applied).

Because deviations in HWE can be due to population demographic changes (Guo and Thompson 1992), we examined additional data from other populations in Alaska for the loci that deviated from HWE. Loci *Edoμ*104, 110, and 122 were found to be in HWE for samples collected near Dutch Harbor ($n = 20$), in the eastern Aleutians, and in Kachemak Bay ($n = 60$) and Montague Strait ($n = 7$) in south central Alaska (for *Edoμ*104 only). This suggests that PWS might not be in mutation-drift equilibrium. However, loci *Edoμ*4, 6, 125, and 216 also deviated from HWE in all populations examined.

The microsatellite markers developed for *E. dofleini* yielded sufficient diversity for potential use in studies that (1) identify individuals and assess kinship; (2) assess population structure and identify stocks; and (3) provide a greater understanding of ecological and evolutionary dynamics.

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