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Microsatellite loci for studies of the common cuttlefish, *Sepia officinalis*.

Niall J. McKeown and Paul W. Shaw

Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Penglais, Aberystwyth, SY23 3DA, UK

e-mail: njm2@aber.ac.uk

Abstract The common cuttlefish, *Sepia officinalis*, represents a model mobile marine species for studies of evolutionary processes occurring across a range of spatial and temporal scales. Such studies are relevant to sustainable fishery management and biodiversity conservation as the species is intensely exploited and an important component of inshore marine ecosystems. The isolation and preliminary characterisation of 10 dinucleotide repeat microsatellite loci is described. Genotyping of 48 adults from Agon Coutainville (France) revealed polymorphism at 9 of the loci. Among the polymorphic loci there was an average of 8.8 alleles per locus (range 3-14) with observed and expected heterozygosity per locus ranging from 0.104 to 0.792 and from 0.101 to 0.806, respectively. No evidence of linkage disequilibrium was detected between any locus pair. The loci described here represent a valuable addition to the suite of genetic markers applicable to *S. officinalis* research.

Keywords Cephalopoda - marine invertebrate – population – behaviour – conservation – management

The common cuttlefish, *Sepia officinalis*, offers intriguing opportunities for studying the evolution of complex mating behaviours (Hanlon et al. 2005) and also represents an ideal model species for studies of population structuring processes (e.g. in response to historical climate changes – Perez-Losada et al. 2007). Furthermore, as the species is intensely exploited throughout much of its range and is an important component of marine ecosystems, such studies are directly relevant to sustainable fishery management and the conservation of marine biodiversity. Shaw & Perez-Losada (2000) developed 7 microsatellite loci for *S. officinalis* which provided insight into species biocomplexity (Perez-Losada et al. 2002), but some loci exhibited non-desirable technical features (interlocus linkage, complex mutation processes). The development of additional loci to enhance genetic studies of *S. officinalis* is reported here.

A partial genomic library was probed for TG and GA dinucleotide repeat sequences following Shaw & Perez-Losada (2000). Hybridising 'positive' clones were sequenced and PCR primers designed from microsatellite flanking regions following McKeown & Shaw (2008). PCR conditions were optimised for 10 loci which were subsequently genotyped in 48 adults collected off the coast of Agon Countainville (France). For each locus the respective forward primer was 5' labelled with a fluorescent dye (Applied Biosystems). Each locus was individually amplified in a 10µl reaction mixture containing ~100 ng of template DNA, 1X buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 pmol of each primer, and 0.2 U of *Taq* DNA polymerase (Bioline, UK). PCR amplifications involved an initial denaturation step (95 ^{o}C for 3 min) followed by 35 cycles of 30 s at 95 ^{o}C, 30 s at the locus optimal annealing temperature (T_a -Table 1) and 30 s at 72 ^{o}C. PCR products were size separated using

an AB3500 DNA sequencer (Applied Biosystems) with allele inference performed using the GENEMAPPER software (version 4.1, Applied Biosystems).

All loci generated high quality products. Allelic variation was detected at all but one locus (*Sof*-di-1) with allele sizes differing by expected multiples of their repeated motifs. Standard diversity indices for each locus, calculated in FSTAT (Goudet 1995), are presented in Table 1 along with primer sequences and allele size ranges. Tests for linkage disequilibrium (LD) and deviations of genotype proportions from expectations of Hardy–Weinberg equilibrium (HWE) were performed using default parameters in GENEPOP 4.0 (Rousset 2008). No significant LD was detected between any locus pair. Genotype proportions conformed to HWE expectations for 8 of the 10 polymorphic loci, with significant departures to HWE due to an excess of homozygotes found at the loci *Sof*-di-4 and *Sof*-di-13 (Table 1).

The loci described here represent a valuable addition to the suite of microsatellite markers available for studies of *S. officinalis*. The variable levels of polymorphism across loci will permit testing of hypotheses relating to phylogeography, population genetics and kinship in this species. Thus, these loci will potentially provide information vital to the design of sustainable fisheries management plans and conservation of the species long-term evolutionary potential.

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Table 1. Primer sequences and characteristics of 10 microsatellite loci developed for *Sepia officinalis*, including repeat motif observed in the clone used to develop each locus (see GenBank accession number) and optimal annealing temperature (T_a) for each primer pair. Allele numbers (N_a) and size range in base pairs (bp), observed (H_O) and expected (H_E) heterozygosity and *P*- values for tests of fit to Hardy-Weinberg equilibrium genotype proportions (P_{HW}) calculated from analysis of 48 individuals.

Locus/ GenBank Accession no.	Primer Sequences (5'-3')	Repeat motif in cloned allele	T_a (^{0}C)	N_a	Size range (bp)	H_O	H_E	P_{HW}
Sof-di-1 KJ169445	F: CAAACAACGTAGACAAAGAAATGG R: TGACCAGGACGAGAGAATGC	$(GT)_{12}$	62	1	198	0	0	-
Sof-di-2 KJ169446	F: AGTCAGCAGCTCGACAAACTC R: TGGAAGTAGCAAGGAAAGCAG	$(GT)_9GA(GT)_3GA(GT)_9$	60	3	168-172	0.104	0.101	1
Sof-di-3 KJ169447	F: ATTGTGATGAAGGCCCAATC R: CTTTCAGACGTCTCACTACATGC	$(GT)_{12}ACAT(GT)_3...(GT)_{18}$	59	11	220-228	0.771	0.758	0.4482
Sof-di-4 KJ169448	F: AATCTTCCACACGGAAGTAAAG R: ACATTGGTCGCAGTCGAAAC	$(GT)_{28}$	58	14	138-188	0.438	0.806	>0.0001

Sof-di-6 KJ169449	F: GGTTTCACCGTCATGTTGTG R: CGAAAGGAAAAGAAGAGACTCG	$(GT)_{14}CT(GT)_5$	58	14	202-252	0.75	0.697	0.624
Sof-di-8 KJ169450	F: TGTATGTCCGAATCACATCG R: AATCGATAGACCGCCTAAACC	$(GT)_{16}$	57	9	148-170	0.646	0.748	0.259
Sof-di-13 KJ169451	F: TGAAAAAGTTCCCCAAAACTTC R: TCCGTACAACCGACGTAGAAC	$(AT)_5AC(AT)_5(GT)_2AT(GT)_{11}$	59	6	194-210	0.319	0.639	>0.0001
Sof-di-14 KJ169452	F: CGATTAGGCGAACCTATCTACG R: ATTCTTTTTATTATTAAGGAAATGCTG	$(GT)_{12}$	61	5	188-204	0.125	0.121	1
Sof-di-21 KJ169453	F: CCAGATTTCTTAAAAACAGTTTCTCTC R: CGAATCGTTTAAAATTTAGTGCTG	$(TC)_4\ldots(CA)_{24}TA(CA)_3(CT)_2TT(CT)_7$	59	12	214-252	0.792	0.792	0.279
Sof-di-26 KJ169455	F: AATATGGGCATATAAGTGTCT R: GCACATAGCCTGACGATTGA	$(CA)_3C(CA)_{16}(TA)_8$	57	5	216-232	0.646	0.635	0.710