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PRIMER NOTE

Characterization of microsatellite markers in the squid, *Loligo bleekeri* (Cephalopoda: Loliginidae)

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Abstract

***Loligo bleekeri* has a long spawning season and the size of mature males changes during the season: dimorphic (large/small) early in the spawning season and monomorphic (small) later in the spawning season. To understand how copulatory behaviours relate to the dimorphism, we developed five polymorphic microsatellite loci in *L. bleekeri*. The level of polymorphism ranged from 10 to 22 alleles with expected heterozygosities ranging from 0.79 to 0.93, suggesting that the novel polymorphic loci should be useful for parentage analysis of *L. bleekeri*.**

Keywords: cephalopod, *Loligo bleekeri*, microsatellite, reproductive strategy

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Loligo bleekeri occurs widely off the coast from southern Korea to northern Japan, where commercial fisheries exploit the spawning aggregations. The spawning season of *L. bleekeri* extends over 6 months and the size of mature males changes during the season: dimorphic (large/small) early in the spawning season and monomorphic (small) later in the spawning season (Natsukari & Tashiro 1991). Before maturation, female Loliginidae store sperm in their seminal receptacles after 'head to head' copulation. Just before spawning, they receive spermatophores during 'male parallel' (Drew 1911) and 'sneaking' copulation (Hanlon *et al*. 1994; Hanlon 1996). Loliginidae have three copulatory behaviours relating closely to male body size and a female has sperm of several males available during spawning. Parentage analysis is a valuable tool to understand complex reproductive relations. Microsatellite markers have been isolated in other loliginid squid (Shaw 1997; Reichow & Smith 1999; Emery *et al*. 2000; Maxwell *et al*. 2000) demonstrating multiple paternities both within clutches and within egg capsules (Shaw & Boyle 1997; Buresch *et al*. 2001; Emery *et al*. 2001). However, the relation to copulatory behaviour is unclear. Furthermore, no microsatellite loci have been isolated in loliginid species living in the western Pacific Ocean. Here, we report the characterization of five microsatellite markers in *L. bleekeri* to understand the copulatory behaviours relating to the dimorphism of body size.

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Adult squid were caught in the coastal water off southern Hokkaido and stored at –20 °C. Gill tissue from each specimen was homogenized by overnight incubation at 50 °C with a solution of 100 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 7.5), 1.4 M NaCl, 2% cetyltrimethyl ammonium bromide, 0.2% 2-mercaptoethanol and 150 μg/mL proteinase K and total DNA was extracted with ethanol-precipitated phenol–chloroform (Munehara & Takenaka 2000). For polymerase chain reaction (PCR) primer design, 150–300-bp fragments digested with *Hae*III were selected by agarose gel electrophoresis and purified using a Gel Band Purification Kit (Pharmacia Biotech). The DNA fragments were ligated into the plasmid vector Blue-script and transformed into *Escherichia coli* XL1B; recombinant colonies were screened with $(GT)_{15}$ and $(GA)_{15}$ oligonucleotide probes that had a 3′-terminal labelled with digoxigenin (Boehringer Manheim). Recombinant DNA fragments were sequenced on an automated sequencer (Gene Rapid; Pharmacia Biotech) following the manufacturer's recommendations. The PCR primer pairs were designed for five loci.

The PCR reaction mixes contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.19 mM of each dNTP, 3.3 μM of each primer, 1.5 U *Taq* polymerase (Takara), 30 μg template DNA and water to a final volume of 6.8 μL. The PCR reactions were performed for 60 s at 94 °C and then 28–30 cycles of 30 s at 94 °C, 50 s at the

Table 1 Primer sequences and characterization of five microsatellite loci isolated from *Loligo bleekeri*

Locus	Repeat unit	Primer sequence (5′–3′)	T_a (°C)	Size range (bp)	No. of individuals	No. of alleles	H_O	H_E	DDBJ Accession no.
Lb1	$(AC)_{16}CC(ACACAG)_3(AC)_7$	TATGCGTTACACTACCACCT ACGATAACCATTACACGACG	57	144–172	32	14	0.94	0.82	AB100368
Lb2	$(GT)_5(GA)_{15}$	TCTTAATTGAACGCCAGATT CTCGAGGAAACTATTTAAACT	51	138–172	32	17	0.88	0.88	AB100369
Lb3	$(TC)_{14}$	GCCATCCGAACAAACTTTAT GTTGCTATCAGCGTCCATTT	51	127–175	32	22	0.94	0.93	AB100370
Lb4	$(GA)_7TA(GA)_6$	CCACGTTGTTCCATGTGTTA CCGAGGGCTTGGTAAATATA	52	158–180	32	11	0.88	0.79	AB100371
Lb5	$(TC)_{13}(TA)_{12}(CA)_4$	TTTTGACATGGTGCCGCGAT ATATGCCCCTCTTTGCTTGC	60	104–128	32	10	0.84	0.81	AB100372

T_a, Annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity.

annealing temperature (Table 1) and 80 s at 72 °C with a GeneAmp PCR system 2400 thermocycler (Perkin-Elmer). The PCR products were electrophoresed in 7% polyacrylamide gel and visualized by the silver-staining method (Tegelström 1986). The size of the PCR products was estimated with 10-bp ladder markers and PCR products from the individual used for primer design.

The results of polymorphism and heterozygosity at each locus within 32 randomly selected individuals are presented in Table 1. The number of alleles ranged from 10 to 22 and expected heterozygosities ranged from 0.79 to 0.93. These microsatellite loci should be helpful for the study of the reproductive system of *L. bleekeri*.

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