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Microsatellite loci from Lanyu scops owl (*Otus elegans botelensis*) and their cross-species application in four species of strigidae

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Abstract

We developed six new microsatellite markers containing tetranucleotide repeat motifs (GATA/CTAT) for Lanyu scops owl (*Otus elegans botelensis*) from an enriched partial library. All these loci are polymorphic and conform to Hardy–Weinberg equilibrium. We cross-species tested these and 12 other microsatellite primer pairs previously developed from *O. elegans* on four other species of owls (*O. lettia*, *O. spilocephalus*, *O. scops*, and *Ninox scutulata*). Results showed that the degree of polymorphism decreased with increasing phylogenetic distance to *O. elegans*. Most loci (66.7, 83.3, and 100%) were polymorphic in the three *Otus* owls but only five (27.8%) were polymorphic in *N. scutulata*. These microsatellites should be very useful genetic markers in studying the mating system, population genetics, and conservation of other little studied Old World *Otus* owls.

Microsatellite molecular marker system has gained great importance in studies as diverse as population ecology, genetic mating systems, relatedness analyses, population differentiation, effects of genetic similarity or inbreeding on fitness, and evaluation of conservation units (DeSalle and Schierwater 1998; Beaumont and Bruford 1999; Pemberton et al. 1999). Microsatellite has many advantages, including being highly polymorphic, easy to score, and in accordance with Mendelian inheritance; before enough polymorphic loci can be identified for use, however, researchers usually must conduct a labor-intensive and cost expensive cloning procedure. Furthermore, a large number of microsatellite loci are usually needed in order to reach better resolution in relatedness analyses (Blouin 2003). Exploring the cross-species applicability of existing microsatellite primers is therefore an advisable and economical first step for a study. In Strigidae, as far as we know, microsatellites have only been developed for *Athene cunicularia* (Korfanta et al. 2002), *Bubo bubo*, (Isaksson and Tegelström 2002), *Strix occidentalis lucida* (Thode et al. 2002) and *Otus elegans botelensis* (Hsu et al. 2003). Only *B. bubo* primers were cross-species tested in *Aegolius funereus* with limited success (Beheim et al. 2002). In this paper, we report the primer pairs for six novel tetranucleotide microsatellite loci isolated from *O. e. botelensis*, and the results of cross-species testing of 18 pairs of *O. e. botelensis* microsatellite primers on four other species of owls, namely collard scops owl (*O. lettia*), mountain scops owl (*O. spilocephalus*), oriental scops owl (*O. sunia*), and brown hawk owl (*Ninox scutulata*).

The *O. e. botelensis* is a small Old World *Otus* owl endemic to Lanyu Island (22° N, 121°5′ E), a 45.7 km^2 island 60 km southeast of Taiwan. We have been studying this subspecies since 1985 (e.g. Severinghaus and Rothery 2001). Since 1999, we

collected about 20 *u* l blood from each bird caught during routine banding by brachial venipuncture and preserved the blood in Queen's lysis buffer (Seutin et al. 1991). We extracted DNA from blood using a method modified from Gemmell and Akiyama (1996), and analyzed the characteristics of the microsatellite loci with DNA samples from 100 adult owls with unknown relationship.

We sequenced plasmid DNA from an enriched partial library constructed in a previous study (Hsu et al. 2003) and found six additional clones containing GATA/CTAT repeat motif. We designed primers for these microsatellites according to the sequences flanking the repeat motifs, using FastPCR 2.3.10 software (available at http://www.biocenter.helsinki.fi/bi/bare-1_html/oligos.htm). After optimizing the polymerase chain reaction (PCR) condition, we labeled each forward primer with HEX, FAM, or TAMRA fluorescent dye.

We amplified each sample under PCR in a 10 μl reaction volume containing about 30 ng genomic DNA, 0.3 μM each primer, 0.5 mM dNTP, 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 0.01% (w/V) gelatin, 0.1% Triton X-100, 0.4 U Pro Taq DNA polymerase (Protech) and 2.0 or 2.5 mM $MgCl_2$ (see Table 1). PCR conditions included a denaturing period of 3 min at 94° C, followed by 34 cycles of 94° C for 30 s, the optimal annealing temperature for each primer pair (as given in Table 1) for 30 s, and 72° C for 30 s, then ending with a final extension period of 3 min at 72° C. We used iCycler thermal cycler (Bio-Rad) for all the PCR work, and MegaBACE 1000 autosequencer (Amersham Biosciences) to electrophorese the PCR products. We analyzed the sizes of alleles with the software Genetic Proflier 2.0 (Amersham Biosciences), and examined the characteristics of these loci with CERVUS 2.0 software (Marshall et al. 1998). A test for linkage disequilibrium of the six novel loci, as well as the twelve loci previously published was conducted using FSTAT 2.9.3.2 (Goudet 2001).

For cross-species application tests, we extracted DNA from blood samples of 23 *O. spilocephalus*, 37 *O. lettia* and 15 *N. scutulata* that came from the Government Office of Stan-Hsiang, Miaoli County, Taiwan. These birds were accidentally caught in a pest control program. Blood samples of 27 *O. sunia* came from the Wildbird Rescue Center of Wild Bird Society of Taipei, Taiwan, the Beijing Raptor Rescue Center, China,

Table 1. Characterization of six novel microsatellites for the *Otus elegans botelensis* ($N = 100$)

Locus	Repeat motif	Primer sequences (5′-3′)	Size of cloned allele (bp)	Number of alleles	Ta^a(°C)	$MgCl_2$(mM)	H_O^b	H_E^c	GenBank accession number
Oe029	$(GATA)_{12}$	F-TGTCCCAACTCCCCTGG R-TTTCCAGAAGCAACCTG	134	5	61	2.5	0.580	0.635	AY536749
Oe081	$(GATA)_{14}$	F-GTAAGGGAAGTAGACGTCTGTTGG R-CAACTTTGTGTCATCTGAAAGG	204	8	61	2.0	0.770	0.750	AY536750
Oe084	$(GATA)_{15}$	F-GGGCATAGTTAGACCTTTGCAG R-CACATCTGTTGTTCTCCGTGTTACC	202	8	64	2.5	0.770	0.775	AY536751
Oe085	$(GATA)_{12}$	F-TGCACAGAAATGAAGAAGAG R-GGGTTTCTCAAAACAGGCAGG	161	5	61	2.5	0.660	0.618	AY536752
Oe129	$(GATA)_{14}$	F-GTCACCTCTTGACATCCGAGTAGC R-GCTAAGAGTCCATTTGCCCATCTG	255	6	65	2.5	0.720	0.770	AY536753
Oe149	$(GATA)_{17}$	F-CACACATCCATTTTGGGGTGC R-GGATGCTGGAACTGACCTGC	217	5	65	2.5	0.780	0.683	AY536754

[a]Annealing temperature. [b]Observed heterozygosity. [c]Expected heterozygosity.

Table 2. *p*-Value for test of linkage disequilibrium in the 18 microsatellite loci of *Otus elegans botelensis* ($N = 100$)

Locus	Oe050	Oe053	Oe022	Oe092	Oe128	Oe045	Oe171	Oe142	Oe054	Oe2–57	Oe3–21	Oe085	Oe3–7	Oe029	Oe149	Oe081	Oe084	Oe129
Oe050	–																	
Oe053	0.0272	–																
Oe022	0.959	0.504	–															
Oe092	0.9048	0.3606	0.5035	–														
Oe128	0.0835	0.897	0.8629	0.9621	–													
Oe045	0.727	0.0395	0.3606	0.4652	0.3521	–												
Oe171	0.884	0.8285	0.0953	0.3147	0.3121	0.3414	–											
Oe142	0.7903	0.5361	0.6189	0.0431	0.8356	0.7269	0.0278	–										
Oe054	0.745	0.338	0.9817	0.4778	0.4244	0.2652	0.7602	0.7644	–									
Oe2–57	0.7549	0.748	0.0171	0.0058	0.6269	0.1528	0.8167	0.0044	0.3405	–								
Oe3–21	0.5082	0.2257	0.5424	0.5863	0.0828	0.5463	0.5567	0.1986	0.8263	0.9032	–							
Oe085	0.8923	0.6801	0.806	0.0446	0.3762	0.6988	0.7462	0.7126	0.0069	0.0146	0.1512	–						
Oe3–7	0.8994	0.5277	0.3248	0.2667	0.9293	0.6144	0.7571	0.4371	0.6503	0.8852	0.1263	0.6216	–					
Oe029	0.8207	0.1654	0.9823	0.0075	0.1846	0.0761	0.5267	0.2699	0.8682	0.7782	0.5283	0.7759	0.4284	–				
Oe149	0.2507	0.1472	0.5251	0.0041	0.2186	0.2714	0.5043	0.2337	0.7979	0.6319	0.5055	0.1063	0.145	0.1373	–			
Oe081	0.0635	0.4607	0.0535	0.6793	0.7739	0.4891	0.8982	0.5952	0.4167	0.0644	0.5661	0.8722	0.2627	0.4973	0.2784	–		
Oe084	0.8361	0.1397	0.5575	0.9116	0.1796	0.8031	0.1435	0.7924	0.732	0.3543	0.3363	0.9328	0.2396	0.3028	0.5326	0.5771	–	
Oe129	0.078	0.3229	0.8599	0.9931	0.1029	0.9366	0.6084	0.3422	0.9728	0.0991	0.032	0.9143	0.8539	0.2488	0.7501	0.7166	0.7153	–

[a]After Bonferroni correction, the adjusted *p*-value for 5% nominal level is 0.000327.

and Maoershan Banding Station, Heilungjiang Province, China. For each locus, all the PCR parameters followed the same regime as that set for *O. e. botelensis*.

The characteristics of the six new microsatellite loci for *O. e. botelensis* are listed in Table 1. None of these loci showed significant deviation from Hardy–Weinberg equilibrium. Although *O. e. botelensis* is an island endemic with an estimated population size fewer than 1000 individuals (Severinghaus, unpublished data), all six loci are highly polymorphic, with 5–8 alleles per locus and heterozygosity between 0.58 and 0.78. These characteristics are similar to those found in the 12 microsatellite loci we isolated previously (Hsu et al. 2003). There was no evidence of linkage equilibrium among the 18 loci, after adjusting the significance level for multiple comparisons with sequential Bonferroni correction (Table 2).

The results of cross-species application of these six microsatellite primer pairs and the 12 pairs we isolated before (Hsu et al. 2003) are summarized in Table 2. The degree of polymorphism (number of polymorphic loci, number of alleles per polymorphic locus, and overall observed heterozygosity) of these eighteen loci decreased as the species' phylogenetic relationship to *O. e. botelensis* increased. This is consistent with the decreasing cross-species applicability of swallow (*Hirundo rustica*) and pied flycatcher (*Ficedula hypoleuca*) microsatellite primers at increasing phylogenetic distances (Primmer et al. 1996).

Among the four owl species examined, *O. sunia* is phylogenetically closest to *O. e. botelensis* (Severinghaus, unpublished data). These two species may share a high proportion of sequences flanking the microsatellites with *O. e. botelensis*, so that all the *O. e. botelensis* primers were successfully PCR-amplified in *O. sunia*. In fact, most of these microsatellite loci turn out to be more polymorphic in *O. sunia* than in *O. e. botelensis*, even though we preferentially screened for microsatellites containing longer repeat motifs during enrichment cloning which should have made them more polymorphic in *O. e. botelensis* Table 3. The difference in polymorphism between these two species could

Table 3. Results of cross-species test of microsatellite primer pairs on four species of owls

Locus	*Ninox scutulata*		*Otus lettia*		*O. spilocephalus*		*O. sunia*	
	N_A/N[a]	H_O[b]	N_A/N	H_O	N_A/N	H_O	N_A/N	H_O
Oe3–7	0/15	–	5/36	0.417	8/22	0.727	10/27	0.963
Oe3–21	1/15	–	1/37	–	1/23	–	15/27	0.556
Oe2–57	0/15	–	12/36	0.833	11/23	0.957	23/27	0.815
Oe022	1/15	–	1/37	–	3/23	0.739	8/27	0.259
Oe045	0/15	–	3/37	0.405	6/23	0.913	16/25	0.64
Oe050	1/15	–	1/37	–	1/23	–	2/27	0.037
Oe053	1/15	–	7/37	0.757	5/23	0.652	9/27	0.63
Oe054	0/15	–	6/37	0.676	5/23	0.522	34/27	0.778
Oe092	7/14	0.643	8/36	0.861	5/23	0.13	18/27	0.778
Oe128	3/15	0.267	7/37	0.568	6/23	0.696	13/27	0.778
Oe142	0/15	–	5/37	0.568	7/23	0.696	14/27	0.815
Oe171	0/15	–	0/37	–	5/22	0.5	13/27	0.741
Oe029	4/15	0.4	6/34	0.382	5/23	0.565	14/27	0.815
Oe081	1/15	–	1/37	–	1/23	–	9/27	0.63
Oe084	1/15	–	6/37	0.595	3/23	0.087	8/27	0.704
Oe085	5/15	0.8	8/37	0.919	10/23	0.696	15/27	0.704
Oe129	4/15	0.467	7/37	0.568	8/23	0.783	6/27	0.593
Oe149	1/15	–	1/37	–	5/23	0.696	9/27	0.667
Number of polymorphic loci	5		12		15		18	
Mean number of alleles per locus[b]	4.6		6.67		6.13		13.11	
Overall observed heterozygosity[c]	0.427		0.630		0.639		0.660	

[a]Number of alleles/number of individuals successfully genotyped. [b]Only polymorphic loci were averaged. [c]Only polymorphic loci were calculated.

result from the fact that *O. sunia* is widely distributed in East Asia with a large and relatively continuous population (our *O. sunia* samples came from locations spanning ca. 24° in latitude), while *O. e. botelensis* population is small and isolated on Lanyu. The effect of genetic drift is usually more pronounced in small populations.

Sixty-seven of the world's 212 species of owls belong to the genus *Otus*, and 41 of them are Old World species (König et al. 1999). Many *Otus* owls in East Asia are found only on islands and their population genetics have rarely been studied. The general applicability of microsatellites developed from *O. e. botelensis* to other *Otus* owls should proof highly useful when studying the genetic diversity, population differentiation, or conservation of Asian *Otus* owls.

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