 doi: 10.1111/j.1471-8286.2005.01108.x

PRIMER NOTE

Ten polymorphic microsatellite markers in the wandering albatross *Diomedea exulans*

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Abstract

We describe 10 new variable dinucleotide microsatellites in the wandering albatross *Diomedea exulans*, as well as conditions for multiplexing and simultaneous genotyping sets of loci. Their variability was assessed in two and one populations from the Crozet and Kerguelen archipelagos (southern Indian Ocean), respectively. Two to 13 alleles were detected per population, and the mean gene diversity was around 0.4. The low genetic differentiation suggests that these populations constitute a single panmictic unit. Cross-species amplification provided some variability at three and five loci in two other marine birds (*Bulweria bulwerii* and *Pagodroma nivea*), but none in *Calonectris diomedea*.

Keywords: albatross, *Diomedea exulans*, microsatellites

Received 16 May 2005; revision accepted 09 June 2005

The wandering albatross *Diomedea exulans* is a long-lived, monogamous and highly philopatric bird exhibiting slow demography (Jouventin & Dobson 2002). It is distributed around the Antarctic over a few archipelagos of the Austral Ocean and has a limited population size that markedly decreased over the last three centuries due to human activities (Weimerskirch *et al*. 1997). Burg & Croxall (2004), using microsatellite markers, showed limited differentiation among populations at the scale of the distribution area. This was confirmed for populations from the Crozet archipelago. However, these loci showed limited polymorphism and some had rather high frequencies of null alleles (J. Bried, M. Nicolaus, P. Jarne, M.-P. Dubois & P. Jouventin, unpublished data). This is a serious limitation when studying intrapopulation structure (e.g. for investigating extra-pair paternity). This prompted us to develop new polymorphic microsatellite markers in *D. exulans* using DNA from an individual sampled in Pointe Morne (Kerguelen).

Bird DNA was extracted from individual blood samples using the QIAmp Tissue Kit (QIAGEN). Isolation of microsatellite loci from an enriched (TC_{10} and TG_{10}) library was performed following Dutech *et al*. (2000), using biotin-labelled microsatellite oligoprobes and streptavidin-coated magnetic beads, with slight modifications: (i) there was no size-selection after digestion with restriction enzyme; (ii) XL10 Gold ultracompetent cells (Stratagene) were used; (iii) recombinant clones were screened with TC_{10} and TG_{10} and AGE1 (AAACAGCTATGACCATGATTAC) or AGE2 (TTGTAAAACGACGGCCAGTG) oligonucleotides using a modified polymerase chain reaction (PCR) method (Waldbieser 1995); (iv) the PCR reagents were 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01%(v/v) Tween 20, 1 μM of each primer, 1.875 mM $MgCl_2$, 1 mM dNTP and 0.75 U of *Taq* DNA polymerase (Eurogentec Red GoldStar) and (v) the PCR programme was 2 min denaturation at 94 °C, followed by 23 cycles (94 °C for 30 s, 60 °C for 1 min, 72 °C for 2 min), and then 5 min at 72 °C.

In all, 1248 clones were screened, 254 of which gave a positive signal. A total of 132 positive clones were sequenced using an ABI PRISM 310 sequencer (Applied Biosystems). Ninety-four sequences included both repeated region and flanking regions allowing determination of PCR primers. Those 15 loci with the largest number of uninterrupted repeat stretch were retained for further amplifications. PCRs were carried out separately for each locus and then multiplexed for two subsets of loci (6A3, 12H8 and 11F3; 7D8, 12C8, 11H7, 10C5 and 11H1) in 10 μL final volume including 0.2 μM of each primer (Table 1) and 1.5 μL of genomic DNA and using the QIAGEN multiplex PCR kit. PCRs were conducted using a PTC100 thermocycler (MJ Research) under the following conditions: 15 min activation of the HotStartTaq DNA polymerase at 95 °C, 30 cycles including 30 s initial denaturation at 94 °C, 90 s annealing

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Table 1 GenBank Accession nos, annealing temperatures in °C (T_a), forward (F) and reverse (R) primers (from 5′–3′) sequences and repeated motif for 10 microsatellite loci characterized in *Diomedea exulans*. For each locus, the allelic size range (SR) and number of alleles (*N*) are given over all populations, and the observed (H_O) and expected (H_E) heterozygosities are given per population (PM, Pointe Morne; PB, Pointe Basse; BM, Baie du Marin). *P* is the probability associated with Hardy–Weinberg permutation test. Mean H_O and H_E over loci are given per population on the last row

							PM			PB			BM		
Locus	Accession no.	T_a	Primer sequence	Repeated motif	SR	*N*	H_E	H_O	*P*	H_E	H_O	*P*	H_E	H_O	*P*
7D8	AY945757	58	hexF: CACGCTGCACTTTGTGATTT R: ACACGGTTTGATTCCTCTGC	$(GA)_{11}$	247–249	2	0.142	0.150	0.543	0.224	0.250	0.621	0.185	0.200	0.570
12C8	AY945754	58	6-famF: TGCATGCCACCAAATCTAAG R: AGCTCAGGACAGCATCACTG	$(GT)_{14}A(GT)$	212–220	5	0.599	0.550	0.338	0.555	0.500	0.321	0.531	0.579	0.636
11H7	AY945753	58	hexF: GCAAGCTAAGTTGGGGGTAA R: ACAGTAGGGCTGCTGCAGAT	$(AC)_{16}$	125–145	10	0.814	0.850	0.639	0.800	0.900	0.909	0.776	0.850	0.795
10C5	AY945750	58	6-famF: TGGAGATGCAATTGCCTAGA R: CAGATGAGGTTTTGGCCAGT	$(GA)_{11}(GC)_2GT\ (GC)_2$	175–179	3	0.050	0.050	0.500	0.000	0.000	—	0.145	0.150	0.541
11H1	AY945752	58	nedF: ACCGGAGCACCAAAATACCT R: CCGGTATTGACCCAGAGAGA	$(GT)_3(GA)(GT)_3(GA)_{16}$	163–187	13	0.876	0.900	0.599	0.886	0.900	0.562	0.899	0.800	0.080
6A3	AY945748	58	6-famF: CCAAGCATCAGGTATTTCAGTC R: TTTTTGCTTTCAGAAAATGAAGG	$(GT)_{10}AAGC(CT)_6$	158–160	2	0.142	0.150	0.531	0.050	0.050	0.050	0.224	0.250	0.613
12H8	AY945756	58	hexF: CATGCCTGAATGCACTTTGT R: CACAATGGGTATAAGATTTTGCTG	$(GT)_7(AT)_7$	169–177	5	0.376	0.250	0.048	0.387	0.450	0.856	0.236	0.150	0.020
11F3	AY945751	58	6-famF: TTTCCAGCACATTCCAACAA R: GTTCAGCCAGAAAAGCAAGC	$(GA)_{10}(GT)_5CT(GT)_6$	230–238	3	0.224	0.250	0.621	0.050	0.050	0.500	0.145	0.150	0.535
6F12	AY945749	56	nedF: ATGAGGAGGAGATGCGAATG R: TTGGGAGTTGTTGGGTTTTT	$(AC)_9GC(AC)_6$	180–182	2	0.296	0.150	0.037	0.450	0.450	0.495	0.328	0.400	0.793
12E1	AY945755	54	6-famF: GCTGGGAGATAAGCAGCAGT R: CCACAGAAGATTTTTTGAG	$(TG)_{11}$	214–224	5	0.735	0.700	0.339	0.582	0.500	0.152	0.694	0.650	0.337
							0.415	0.425		0.389	0.399		0.406	0.416	

 Molecular Ecology Notes, 10.1111/j.1471-8286.2005.01108.x

Table 2 Cross-species amplification of 10 *Diomedea exulans* microsatellite loci in three petrel species. P, M and NA refer to polymorphic, monomorphic and no amplification, respectively

Locus	*Bulweria bulwerii*	*Calonectris diomedea*	*Pagodroma nivea*
6A3	P	M	M
11H1	M	M	M
12H8	P	M	P
10C5	P	M	P
11H7	P	M	M
6F12	NA	NA	NA
12E1	M	M	M
12C8	P	M	P
11F3	M	M	M
7D8	NA	NA	NA

(see Table 1 for annealing temperatures) and 60 s extension at 72 °C, and then 30 min final extension at 60 °C. Two microlitres of diluted PCR products (1/40) were pooled in 15 μL of deionized formamide and 0.2 μL GeneScan-500XL ROX Size Standard and analysed on an ABI PRISM 310 Genetic Analyser.

Variability was screened in three populations (20 individuals per population) of *D. exulans*. Two populations are located in the Crozet archipelago and separated by 10 km (Baie du Marin and Pointe Basse), and the third one is from Kerguelen main island (Pointe Morne) which is separated from Crozet by about 1500 km (Table 1). Ten out of 15 loci turned out to be polymorphic (Table 1). Two to 13 alleles were detected per population (Table 1), and the mean number of alleles per locus was 3.8, 3.9 and 4.4 for Pointe Morne, Pointe Basse and Baie du Marin, respectively (Table 1). The gene diversity per locus rose up to 0.90. Although three tests of Hardy–Weinberg were significant at the 5% level, none remained significant after multiple testing. This result suggests that null alleles, if present, have low frequency. No evidence of genotypic disequilibria was detected. Multilocus estimates of F_{ST} were 0.004 (permutation test on individuals, $P = 0.323$; Baie du Marin and Pointe Basse), 0.008 ($P = 0.166$; Pointe Morne and Baie du Marin) and 0.014 ($P = 0.063$; Pointe Morne and Pointe Basse), suggesting that these three populations constitute a single panmictic unit. All analyses were conducted using GENETIX (Belkhir *et al*. 1996–2004).

Cross-species amplification of the 10 *D. exulans* microsatellite DNA primers was carried out in five individuals from each of three other procellariiform species from the same family (*Bulweria bulwerii, Calonectris diomedea* and *Pagodroma nivea*) under the same technical conditions as discussed previously. Some loci exhibited variation in two species, but no discussed previously at all was detected in *C. diomedea* (Table 2).

The loci characterized here are complementary to those of Burg & Croxall (2004). The most polymorphic ones should prove useful for studying kinship and mating systems in the wandering albatross, as well as for detecting migrant individuals that have been otherwise characterized from banding.

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