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PRIMER NOTE

Microsatellite loci for behavioural studies of Eclectus parrot (*Eclectus roratus*: Aves)

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Abstract

Ten polymorphic microsatellite loci were isolated from the cooperatively breeding and sexually dichromatic Eclectus parrot (*Eclectus roratus*). Nine loci were in Hardy–Weinberg equilibrium and unlinked. One locus, Ero1, was presumed to be sex-linked since females, the heterogametic sex, were all homozygous, whereas 72% of males were heterozygous. A DNA database search revealed that Ero8 is probably an independent isolation of the microsatellite locus AgGT83 of the parrot *Amozona guildingii*. With high heterozygosity (0.41–0.89) and number of alleles (two to 13), these loci should prove useful for investigating the mating system of these unusual Australasian parrots.

Keywords: behaviour, *Eclectus roratus*, microsatellite, sexual dichromatism

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Male and female Eclectus parrots look so different that, for a time, they were thought to be separate species (Forshaw & Cooper 1989). Whereas most parrots show relatively little sexual dichromatism, female Eclectus parrots (*Eclectus roratus*) have bright red and blue plumage, whereas the males are mostly green. Current sexual selection theory can explain ornamentation in one sex or similar ornamentation in both sexes, but has difficulty explaining entirely different elaborations in each sex (Andersson 1994). A major puzzle in Eclectus parrots is that unlike other species exhibiting reverse dichromatism, there is no reversal of sex roles (Heinsohn & Legge 2003). We sought microsatellite markers for determining fundamental data on parentage, population structure and dynamics. These data will help us to unravel the mating system and how it relates to the unique form of sexual dichromatism in this species.

DNA was extracted from Eclectus parrot blood samples using an ammonium acetate method (Nichols *et al*. 2000). All samples were collected from individuals in one population of the subspecies *Eclectus roratus macgillivrayi* at Iron Range National Park, Cape York Peninsula, in far north Queensland, Australia (12°45′S, 143°17′E). In general, polymerase chain reactions (PCR) of 20 μL consisted of 0.5 U of *Taq* polymerase, $MgCl_2$ (published concentrations or see below), 10× reaction buffer and dNTPs (200 μM), all supplied by QIAGEN, and 200 nM of each primer. Reactions were run in 0.2 mL microtitre plates or tubes on a Corbett Research PalmCycler thermocycler. Following Scott *et al*. (2001), a random genomic DNA library was created, then transformed colonies were screened and rescreened with radio-labelled probes and positive colonies sequenced on both strands. Approximately 30 000 colonies were screened to reveal 96 positive colonies. These were rescreened, and the 22 confirmed positives were sequenced. Another library was made commercially (Centre for Identification and Diagnostics, University of Queensland) using the enrichment method of Fischer & Bachmann (1998). The 100 colonies provided were all sequenced as above. Primers were designed using PRIMER3 (Rozen & Skaletsky 2000) manufactured (Proligo) for 10 clones from the random library and six clones from the commercial library that contained eight repeats or more and flanking sequence suitable for primer design. One primer in each pair contained a 5′-M13 (TGTAAAACGACGGCCAGT) tail for use in a universal dye-labelling method (Schuelke 2000).

Primer pairs that gave consistent specific products were further tested for polymorphism on 36 individuals chosen on the basis that they were unlikely to be related to others in the sample. Reactions (20 μL) contained an M13 primer

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Table 1 Characterization of microsatellite loci in the Eclectus parrot *Eclectus roratus*.

Locus	Repeat motif	Primer sequence (5′–3′)	$MgCl_2$	N_A	Range (bp)	H_O	H_E	Excl1	Excl2
Ero1	$(CA)_{27}$	TTAATATGGAAAGTTCTCACCC	1.5	10	174–202	0.36	0.74	0.54	0.36
		*TTAGTTGCGAGAATACCAGG				(0.72)	(0.74)		
Ero2	$(CA)_{14}$	CACAAATGCACATATTAACTCC	2.0	3	186–190	0.60	0.62	0.33	0.19
		*AATGTAACATGCCACCTACC							
Ero3	$(CA)_{12}$	ATGTGACAACTACTTTTTTCCC	1.5	2	204–206	0.47	0.49	0.19	0.12
		*GAGACAATCCTCACCAAGC							
Ero4	$(AAC)_{10}$	CAGAATTCAAAAAATCATTGC	2.0	6	241–250	0.58	0.64	0.39	0.23
		*ATGGTCACTCTATTCTTTCTGG							
Ero5	$(CA)_{18}$	CTGAGTGCATATGGCATCAAG	2.0	6	410–430	0.72	0.72	0.48	0.31
		*ATCCCACGCAGACACAACTATCAGAG							
Ero8	$(AC)_{36}$	AGTATGGGAAACATCACAGG	2.0	13	175–199	0.89	0.88	0.77	0.62
		*GCTATTCAGGCAGTGAAAGT							
Ero9	$(TGA)_3T(TGA)_8TTGA$	CATATTTGCCTATCCCACAT	2.0	5	226–259	0.75	0.73	0.51	0.33
		*CCTGTTCCAATGCTTGAC							
Ero10	$(CTAT)_{16}(CTAC)_{10}$	GATGCAAAAGTCTGGAAGAG	2.0	12	180–259	0.81	0.82	0.66	0.49
		*CCTTAGTTGGTAGCCTGTGA							
Ero11	$(CA)_{10}$	ACATCTGCCAAGCAACTTGA	2.0	4	121–129	0.44	0.59	0.33	0.18
		*GTCTCCTGCCATTTCTGACC							
Ero15	$(GAG)_5$	CTGCACATTGTCCTGATGCT	2.0	2	206–209	0.41	0.44	0.17	0.10
		*GGATTTCATTCCGAACTGCT							

All loci were amplified in 36 individuals with the $MgCl_2$ concentrations shown in mM and the same cycling parameters (see text). Listed for each locus is the number of alleles (N_A), allele size range in bp (range), observed (H_O) and expected (H_E) heterozygosities and the probability of excluding a potential parent when one is known (Excl1) or when the other is not typed (Excl2). For the sex-linked locus Ero1, H_O and H_E are also given for males only ($n = 18$) in brackets. The asterisk (*) in the primer sequence denotes a 5′ tail (TGTAAAACGACGGCCAGT), attached to the primer sequence. Sequences have been deposited in GenBank under Accession nos AY921624–AY921633.

(200 nM) 5′-labelled with an ABI dye (VIC, FAM or NED), and the locus-specific tailed (15 nM) and untailed primer (200 nM), 40–100 ng of genomic DNA and $MgCl_2$ concentrations shown in Table 1. All loci were amplified using the same conditions: one cycle at 94 °C for 5 mins, then cycles of 94 °C for 30 s, annealing temperature for 30 s and 72 °C for 45 s, where the annealing temperature was initially 62 °C and was decreased by 3 °C every two cycles down to 50 °C, which ran for 30 cycles, followed by a final step of 72 C for 45 mins. PCR products were electrophoresed on an ABI PRISM 3100 automated sequencer together with a size standard (Liz-500) according to the manufacturer's instructions. Fragment sizes were estimated using the ABI GENESCAN™ fragment analysis software.

Ten loci were found to be polymorphic ($N_A = 2$–13, $H_E = 0.41$–0.89, Table 1). Using the tests implemented in GENEPOP 3.3 (Raymond & Rousset 1995), no pairs of loci showed linkage disequilibrium ($P > 0.05$), and only Ero1 deviated significantly from Hardy–Weinberg (HW) expectations with a highly significant heterozygote deficiency ($P << 0.001$). In this locus, all 18 females were homozygous compared with 28% homozygosity in males. This suggests that this locus is sex-linked since females are the heterogametic sex in birds. Using just the males ($n = 18$), Ero1 conforms to HW expected genotype proportions ($P = 0.58$). The general probability of excluding one parent when the other is known or when the other is not typed (Equations 1a and 2a in Jamieson & Taylor 1997) is given for each locus in Table 1 and is high when applied across all 10 loci ($P = 0.998$ and $P = 0.977$).

Each polymorphic locus was compared for sequence similarity with the entire GenBank database using a BLAST search (http://www.ncbi.nlm.nih.gov/blast) with standard settings for a nucleotide-to-nucleotide search. From this, the clone Ero8 exhibited a very high alignment score (246 bits) with the GT repeat containing locus AgGT83 (Accession no. AF339762), isolated from the St. Vincent parrot *Amazona guildingii* (Russello *et al*. 2001). There are only 10 transitions, three transversions and no indels in the 290 bp flanking the repeated DNA in an alignment done manually. Since this laboratory has never handled *Amazona* DNA or amplified DNA using AgGT83 primers, this represents a remarkable independent isolation of a locus and not contamination.

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