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PRIMER NOTE

Isolation of microsatellite loci in the Capricorn silvereye, *Zosterops lateralis chlorocephalus* (Aves: Zosteropidae)

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Abstract

The Capricorn silvereye (*Zosterops lateralis chlorocephalus*) is ideally suited to investigating the genetic basis of body size evolution. We have isolated and characterized a set of microsatellite markers for this species. Seven out of 11 loci were polymorphic. The number of alleles detected ranged from two to five and observed heterozygosities between 0.12 and 0.67. One locus, ZL49, was found to be sex-linked. This moderate level of diversity is consistent with that expected in an isolated, island population.

Keywords: microsatellite, passerine, silvereye, white-eye, Zosteropidae, *Zosterops*

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The Capricorn silvereye (*Zosterops lateralis chlorocephalus*) has evolved to be up to 40% larger than its ancestor, the continental race *Z. l. familiaris* (Degnan 1993). This species inhabits the islands of the southern Great Barrier Reef, Australia and, because its biology is well known, it provides an ideal population in which to investigate the evolution of body size shifts. The genetic basis of such morphological change can be investigated through new, molecular-marker assisted methods for the estimation of inheritance. Although a set of four polymorphic microsatellites already exists for this species (Degnan *et al*. 1999), additional markers are necessary for the resolution of relatedness amongst individuals required for the application of molecular-marker methods.

To construct a microsatellite library, DNA was extracted from the liver tissue of a single individual using a cetyltrimethyl ammonium bromide protocol (Graham *et al*. 1994). A genomic library was constructed and screened using a protocol modified from the method of Fisher & Bachmann (1998). Library construction involved the digestion of genomic DNA with *Rsa*I restriction enzyme (Roche), the ligation of 21-mer (5′-CTCTTGCTTACGCGTGGACTA-3′) and 25-mer (5′ phosphate-TAGTCCACGCGTAAGCAAGAGCAC-3′) *Mlu*I adaptors to the digested genomic DNA and amplification by polymerase chain reaction (PCR). The amplification products were denatured and hybridized to 19 single-stranded biotin-labelled (5′ end) oligonucleotides, which were then captured by Streptavidin-coated magnetic beads (Roche). The oligonucleotides had the following motifs: $(CT)_{15}$, $(CA)_{20}$, $(CAT)_{14}$, $(ACA)_{14}$, $(CTG)_{10}$, $(ACT)_{14}$, $(AGA)_{14}$, $(CTA)_{14}$, $(CTT)_{14}$, $(GAC)_{10}$, $(CAG)_{10}$, $(AGC)_{8}$, $(ACC)_{8}$, $(AAC)_{8}$, $(TAT)_{8}$, $(CCCT)_{6}$, $(CTAG)_{6}$, $(GATA)_{6}$, $(GACA)_{6}$. The enriched genomic products were eluted from the magnetic beads and amplified again using the *Mlu*I adaptors. The amplified products were restricted with *Mlu*I enzyme (Roche) to remove the *Mlu*I adaptors. Prepared products were then cloned into pUC19 vector (Stratagene) and transformed into Max Efficiency DH5α™ competent bacterial cells (GibcoBRL). Two hundred clones were amplified with the pUC⁻ and pUC⁺ primers (5′-CAGGAAACAGCTATGACC-3′ and 5′-GTTTTCCCAGTCACGACG-3′, respectively). Amplified products were sequenced with pUC⁻ primer to detect microsatellite presence in clones. Clones containing microsatellite repeats and flanking regions suitable for primer design were sequenced in the reverse direction with the pUC⁺ primer. Thirteen consensus sequences were submitted to the European Bioinformatics Institute's EMBL database (Accession nos AJ517993–AJ518005). The sequences were confirmed unique in the EMBL and GenBank databases using BLASTN 2.2.4 software (Altschul *et al*. 1997) and primer pairs were designed by eye. To reduce nonspecific amplification (Brownstein *et al*. 1996), a GTTT 'pigtail' was added to the

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Table 1 Characterization of seven polymorphic microsatellite loci in the Capricorn silvereye, *Zosterops lateralis chlorocephalus*

Locus	EMBL Accession no.	Repeat motif (5′–3′)*	Primer sequence (5′–3′) and 5′ primer modification (underlined)	T_a (°C)	$MgCl_2$ (mM)	n	No. of alleles	Obs. allele size range (bp)	H_O	H_E
ZL41	AJ517994	$(GACA)_7$	F: NED-TATGGAGAAGTGTAAGAAAGG R: GTTT-CGACCAAAGTGCAGTTATC	53.0	1.5	45	5	82–92	0.60†	0.69
ZL44	AJ517996	$(CT)_9$	F: HEX-CTGTCCCTGCCCTCTCATC R: ACCATGGCAGAGGCACCAA	53.0	1.0	39	3	220–229	0.39	0.39
ZL45	AJ517997	$(GACA)_5$	F: 6-FAM-CCGGAGCACCACGCACAGC R: GTTT-GGGTCCAAGCGCCTCGAG	57.5	1.0	49	2	108–116	0.12	0.19
ZL46	AJ517998	$(GTT)_7$	F: GTCAGTGCTGTGCTTTGAT R: HEX-AACCTGAAATTACACTTCT	54.2	1.5	52	4	127–150	0.42	0.46
ZL49	AJ518000	$(GTCT)_7$	F: TGTCCTCGTGCAAGATGG R: NED-C-TTGCAAGCTGATGTCTTAT	59.3	1.5	52	4	154–170	0.39‡	0.40
ZL50	AJ518001	$(GCT)_7$	F: NED-T-AAGGTGCCGAGGTCCTGT R: G-TTTGCATGAGTGCATGCTGG	59.3	1.5	46	3	126–132	0.59	0.49
ZL54	AJ518005	$(CA)_{13}$	F: NED-CACGACTTCTCAAGCAGAC R: GAGCCTTGCACAAACGGAC	57.5	1.5	51	4	118–126	0.67	0.62

*From sequenced clone.
†Loci deviating significantly ($P < 0.007$) from Hardy–Weinberg equilibrium. ‡Observed and expected heterozygosities calculated using males only.
n, number of Heron Island individuals tested; T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity.

5′ end of ZL41R and ZL45R primers (Table 1). An extra base (C, T and G, respectively) was added to the 5′ end of ZL49R, ZL50F and ZL50R (Table 1) to prevent these primers annealing to themselves.

Silvereye DNA for PCR was extracted from blood using a salt precipitation method (Nicholls *et al*. 2000). PCR reactions were performed in 10-μL volumes and contained 15 ng of DNA, 1 μM of each primer, 0.2 mM of each dNTP, 0.25 U of DNA polymerase (Thermoprime^{Plus}, ABgene), in the manufacturer's buffer [final concentrations 20 mM $(NH4)_2SO_4$, 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween], including 1.0 or 1.5 mM $MgCl_2$ (Table 1). PCR cycling was performed in a Hybaid Touchdown Thermocycler (Thermo Hybaid) and followed a pattern of initial denaturation for 3 min at 94 °C, followed by 33 cycles of 45 s at 94 °C, 45 s at annealing temperature (T_a, Table 1) and a final 20 s at 72 °C. PCR reaction conditions were optimized for all loci using both a thermal gradient of annealing temperatures (50–60 °C) and a magnesium chloride ($MgCl_2$) concentration gradient (0.625 mM–2.5 mM per reaction). Eleven out of 13 primer sets successfully amplified products of the expected size. One primer in each pair was labelled with one of three fluorescent dyes: HEX™, 6-FAM™ and NED™ (Applied Biosystems) (Table 1). PCR products were analysed using an ABI 377 DNA Sequencer (Applied Biosystems).

Products were initially tested for polymorphism on 20 putatively unrelated individuals from Heron Island, the largest population of Capricorn silvereyes. Seven loci were polymorphic (Table 1) and four were monomorphic (further details are available on the Molecular Ecology Notes database: http://snook.bio.indiana.edu/MENotes/home.html). Polymorphic loci were then characterized using larger sample sizes (39–52 individuals), also obtained from the Heron Island population. Observed and expected heterozygosities were calculated using the program CERVUS version 2.0 (Marshall *et al*. 1998). Departures from Hardy–Weinberg equilibrium and linkage among loci were tested using GENEPOP (http://wbiomed.curtin.edu.au/genepop; Raymond & Rousset 1995). Significant deviations from Hardy–Weinberg equilibrium were found at two loci, ZL41 and ZL49 (P-values ≤ 0.000, Bonferroni $\alpha = 0.007$, $k = 7$), possibly indicating the presence of null alleles at ZL41. No loci were found to be in linkage disequilibrium, but an indication of sex linkage was found for ZL49 ($P = 0.0019$, Bonferroni $\alpha = 0.0018$, $k = 28$). In a sample of 52 individuals genotyped at this locus, and which had had their sex determined molecularly, all females (ZW) were hemizygous ($n = 26$), with 62% of males (ZZ) being homozygous and 38% heterozygous ($n = 16$ and $n = 10$, respectively). These results suggest that ZL49 is situated on the Z chromosome. Once females were removed from the sample, the locus was observed to be in Hardy–Weinberg equilibrium (Table 1).

Moderate to low levels of polymorphism were observed in this set of loci (2–5 alleles in 39–52 individuals). This low level of polymorphism is consistent with that expected from a small, isolated, island population. A similar pattern has been found previously, with this subspecies showing lower levels of genetic diversity than others in the *Zosterops lateralis* complex (Degnan 1993; Degnan *et al*. 1999). Although we have not tested the utility of these primers across many taxa, ZL44 and ZL45 amplify polymorphic loci in the house

sparrow, *Passer domesticus*, and the magpie, *Pica pica*, respectively (N. Ockendon and D. Galvez, personal communication, see the Sheffield Molecular Genetics Facility's primer database for details: http://www.shef.ac.uk/misc/groups/molecol/birdmarkers.html; Dawson *et al*. 2003). The primers we have presented here may prove to have higher levels of diversity in other passerine birds.

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