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Authors

Thomassen, Henri A.
Jones, Kenneth C.
Pollinger, John P.
et al.

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TECHNICAL NOTE

Characterization of novel microsatellite loci for *Myzomela cardinalis* and *M. rubrata* honeyeaters, and cross-amplification in other species

Henri A. Thomassen · Kenneth C. Jones · John P. Pollinger · Thomas B. Smith

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Abstract *Myzomela* honeyeaters are distributed from eastern Austalia and Indonesia throughout the islands of the tropical Pacific, a biodiversity hotspot that is particularly vulnerable due to small population sizes, habitat destruction, and high levels of isolation between islands. We developed fourteen microsatellite loci for *Myzomela* honeyeaters from the tropical Pacific. Microsatellites were tested for polymorphism on a panel of 104 individuals. The number of alleles ranged from 3 to 23, observed heterozygosity from 0.067 to 0.913, and the fixation index from −0.143 to 0.634. Cross-amplification was tested in 18 different species and subspecies in the genus *Myzomela* from throughout the Pacific, Australia, and eastern Indonesian archipelago. These new microsatellites could potentially be useful in making informed conservation decisions regarding *Myzomela* honeyeaters on the islands of the tropical Pacific.

Keywords Microsatellite · Honeyeater · Pacific · Meliphagidae · *Myzomela*

H. A. Thomassen (✉) · J. P. Pollinger · T. B. Smith
Center for Tropical Research, University of California, La Kretz Hall, Suite 300, 619 Charles E. Young Dr. East, Los Angeles, CA 90095, USA
e-mail: hathomassen@ucla.edu

J. P. Pollinger · T. B. Smith
Department of Ecology and Evolutionary Biology, University of California, 621 Charles E. Young Dr. East, Los Angeles, CA 90095, USA

K. C. Jones
Genetic Identification Services Inc., 9552 Topanga Canyon Blvd., Chatsworth, CA 91311, USA

Introduction

The tropical Pacific region constitutes one of the world's 25 designated hotspots of biodiversity (Myers et al. 2000), but at the same time its terrestrial avifauna is threatened in many ways. Population sizes are often small due to limited availability of suitable land surface, human land-use changes are continuing to decrease the original habitat (e.g., Buchanan et al. 2008), and introduced diseases (e.g., Jarvi et al. 2001), predators (e.g., Fritts and Rodda 1998), and non-native competing species greatly impact naïve bird populations (e.g., Steadman 2006). Contrary to traditional views that the islands of the tropical Pacific serve as a sink of biodiversity generated on the mainland, it was recently shown that diversification likely occurs in situ, and lineages that arise in the Pacific may recolonize the Australasian mainland (Filardi and Moyle 2005). Thus, the tropical Pacific constitutes a diverse and threatened region where biodiversity is likely being generated, yet it is relatively understudied.

One of the most widespread bird genera in the Pacific is constituted by the *Myzomela* honeyeaters (Pratt et al. 1987), a monophyletic group within the Meliphagidae (Driskell and Christidis 2004). The genus is distributed from the eastern Indonesian islands and Australia to the Pacific islands of the Commonwealth of the Northern Mariana Islands (CNMI) and American Samoa. Despite the fact that the Pacific species in this genus are still comparatively common, population trends are often negative. For example, a recent study reported a 72% decline of the Micronesian honeyeater (*M. rubrata*) population on Rota, CNMI (Amar et al. 2008). Whereas populations from many islands are often treated as a single species, it is plausible that the *Myzomela* honeyeaters of the Pacific constitute multiple recently diverged taxa, and should be treated as such for conservation purposes.

Here we describe the isolation and characterization of 15 novel tri- and tetranucleotide microsatellite loci from *M. cardinalis* and *M. (cardinalis) rubrata* populations on six islands ($n = 104$) in the northern tropical Pacific: Sarigan, Saipan, Rota (CNMI), Yap, Chuuk, and Pohnpei (Federated States of Micronesia). Blood samples (50–100 µl) were taken from captured birds and stored in lysis buffer. No blood samples were available from the population on Sarigan, in which case we used feather samples for DNA extractions. DNA was extracted using the Qiagen QIAamp DNA Mini Kit using the manufacturer's protocol for either blood or tissue. DNA extractions from blood samples were used to create an enriched library at Genetic Identification Services, Inc. (Chatsworth, California). Details on the enrichment procedure can be found in Jones et al. (2000). In brief, the extracted DNA was incompletely digested using blunt-end restriction enzymes *Bsr*B1, *Eco*RV, *Hae*II, *Pvu*II, *Rsa*I, *Sca*I, and *Stu*I. *Hin*dIII adaptors were attached to fragments of 300-750 bp long. Fragments containing target repeat motifs were captured using 5′-biotinylated probes. Capture molecules with the following repeat motifs were used: AAC, ATG, CATC, and TAGA. Captured fragments were subsequently amplified using a primer complementary to the adaptor, digested with *Hin*dIII and ligated into the *Hin*dIII site of pUC19. Plasmids were electroporated into *E. coli* DH5α, and recombinant clones were selected for sequencing on an ABI 377 sequencer using ABI Prism Taq dye terminator cycle sequencing methodology.

Primers were originally designed for 28 loci using WebSat (Martins et al. 2009). Of these, 15 loci amplified

Table 1 Microsatellite loci developed for *Myzomela* honeyeaters

Locus/genbank acc. no.	Repeat motif	Primer sequence (5′–3′)	N_a	Size range	H_O	H_E	F_{is}
Mr 11	GTT	GTTTGTTTGTTTGTTTGGGG	8	146–167	0.291	0.797	0.634
GU135610		TGCTAAAAGGGGAAGAGACTTA					
Mr 12	CAA	ATATTACAGGAGGCGGGGT	3	153–159	0.067	0.065	−0.030
GU135611		TCCACTCTTTATTCCAACCATC					
Mr 15	GAT	AAACTGAGGTGGGAGCTTG	12	107–140	0.702	0.877	0.199
GU135612		AGGGGTTTGTGCCTCCAT					
Mr 16	ATG	CAGGTTGAGGTAAGAACAGCTT	11	161–200	0.846	0.838	−0.010
GU135613		GTCCAGTAGGTGATGAGCAAAT					
Mr 27	ATCC	AAACCACTGACAAAATGCCTC	10	167–203	0.740	0.799	0.073
GU135614		ACTGAGAGATGGATGGATCAAG					
Mr 28A/B	TCCA	CAACCAACCAACCATCAAAT	15	107–227	0.827	0.903	0.084
GU135615		TATATGGGCATGACTGCGTA		240–284			
Mr 29	GGAT	AGTTGTCAGAGACTTGGAATGG	6	220–240	0.712	0.742	0.040
GU135616		ATTATCTCCTCCTCCTCCTCAC					
Mr 34	CCAT	CTTACCCAAAACCTCCTCCTC	9	227–259	0.904	0.791	−0.143
GU135617		CACTTTCAGCGCAAATTACA					
Mr 35	CATC	TGACAGCAGGTGATGCAG	13	143–191	0.817	0.869	0.059
GU135618		TGTCTATACCTCTCTTTCCTCCT					
Mr 36	CATC	CATCGACCGTTATTTTCACTC	7	155–179	0.654	0.740	0.116
GU135619		CTCTTCTGGACTATGGCCTTG					
Mr 40	(AGAT)ATAG(AGAT)	AGCTGCCCAAATAACTCACA	14	165–233	0.635	0.893	0.289
GU135620		TTCAGGGAATCTCCTGTCAC					
Mr 41	(ATCT)A(ATCT)	TCTCCTGCTATCTCACATCTATCT	12	137–205	0.769	0.791	0.027
GU135621		TTGCCATCTTCTCTGTCTCAC					
Mr 45	TCTA	GGTGCAGTCAAGATGTTTGTT	16	119–183	0.894	0.851	−0.051
GU135622		TTTAAGAGCAGGAGAAGTCAGC					
Mr 47	TATC	AGCACACCTAATGTCCTCCA	23	133–229	0.913	0.918	0.005
GU135623		GCGACATCACACATTTCAGA					
Mr 48	AGAT	GTCCCAAGTGAATTTGCATAG	16	151–211	0.837	0.906	0.077
GU135624		TGTCTTTTCATGTTCCACCAC					

Shown are locus names, GenBank accession number, repeat motif, forward (top) and reverse (bottom) primers, number of alleles (N_a), fragment size ranges, observed (H_O) and expected heterozygosity (H_E), and fixation index (F_{is}). Forward primers contained an additional 16-bp M13-hybrid primer binding site on the 3′ end. Polymorphism was tested in 104 individuals, all of which amplified for the loci tested

Table 2 Cross-amplification of developed microsatellite loci in members of the genus *Myzomela*

Species	Mr11	Mr12	Mr15	Mr16	Mr27	Mr28	Mr29	Mr34	Mr35	Mr36	Mr40	Mr41	Mr45	Mr47	Mr48
M. albigula albigula	−	+	+	+	+	+	+	−	+	+	−	+	+	−	+
M. blasii	−	+	+	+	−	−	+	−	+	+	−	+	+	−	+
M. c. chermesina	+	+	+	+	+	+	+	−	+	+	−	−	+	+	+
M. c. lifuensis	−	+	+	+	+	−	+	−	+	+	−	+	+	+	−
M. c. sanfordi	+	+	+	+	+	−	−	−	+	+	−	+	+	+	+
M. c. tenuis	+	+	+	+	+	−	−	−	+	+	−	+	+	+	−
M. eques eques	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+
M. erythromelas	−	+	+	+	+	−	−	−	+	−	−	−	+	−	+
M. jugularis	+	+	+	+	+	+	+	−	+	+	−	−	+	−	+
M. lafargei	−	+	+	+	−	+	−	−	+	−	−	−	+	+	+
M. malaitae	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+
M. melanocephala	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+
M. nigrita ernstmayeri	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
M. obscura obscura	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+
M. rosenbergii rosenbergii	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+
M. sclateri	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+
M. sanguinolenta sanguinolenta	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+
M. tristrami	−	+	+	+	+	+	+	−	+	+	−	−	+	+	+

Because only a single toe pad sample for each taxon was available and tested, the level of polymorphism could not be evaluated. *M.c.* = *Myzomela cardinalis*

correctly and were polymorphic in 104 individuals. Fragments were amplified by means of the M13-hybrid primer process. In this process, a 16-bp fragment is added to the 5′ end of the forward primer for binding of the dye-labeled M13-hybrid primer (Boutin-Ganach et al. 2001; Schuelke 2000). Primer mixes were prepared as follows: 2 µl reverse primer (100 µM); 4 µl forward primer (2.5 µM); 4 µl 6FAM dye-labeled M13-hybrid primer (2.5 µM); 90 µl RNAse/DNAse-free water. Amplification was carried out in 10 µl reactions containing 5 µl Qiagen Multiplex Mastermix, 0.5 µl BSA (10 mg/ml), 1 µl primer mix, 2 µl RNAse/DNAse-free water, and 1.5 µl template DNA (30–100 ng in total). The following cycling conditions were used: an initial step of 95°C for 15 min; 25 cycles of: 30 s at 94°C, 90 s at 59°C, 60 s at 72°C; 20 cycles of: 30 s at 94°C, 90 s at 53°C, 60 s at 72°C; and 30 min at 60°C. PCR products were run on an ABI 3730 capillary sequencer, and allele sizes were scored manually using GS 500-LIZ size standard in GeneMapper v3.7 genotyping software (ABI).

Tests for deviations from Hardy–Weinberg equilibrium (HWE) for each locus and each population, and calculations of observed (H_O) and expected heterozygosity (H_E) and fixation indices (F_{is}) were carried out in GenAlEx v6 (Peakall and Smouse 2006). Tests for linkage disequilibrium (LD) were performed in Genepop 4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons, no consistent departures from HWE were detected across populations ($P > 0.05$). One of the primer combinations (Mr 28) amplified two separate regions, which were highly significantly linked (Mr 28A and Mr 28B; $P < 0.001$). Among the remaining loci, LD was only suggested in the population from Saipan for Mr 40 and Mr 47. The number of alleles ranged from 3 to 23 (Table 1). Observed and expected heterozygosities ranged from 0.067 to 0.913 and from 0.065 to 0.918, respectively. Finally, F_{is} ranged between −0.143 and 0.634.

Cross-amplification of primers in other species was evaluated in 18 different *Myzomela* species and subspecies. DNA was extracted from toe pads collected from museum material using the Qiagen QIAamp DNA Mini Kit according to the manufacturer's protocol for tissue. Most loci amplified well (Table 2), however, because only a single individual per species was available, polymorphism could not be tested. The microsatellites developed here could be useful in population genetic studies of *Myzomela* honeyeaters, and in the conservation of members of this genus in the tropical Pacific.

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