

PRIMER NOTE

Development of 10 microsatellite loci for Yellow-billed Magpies (*Pica nuttalli*) and corvid ecology and West Nile virus studies

HOLLY B. ERNEST,*† JAY A. WELL* and JENNIFER D. KURUSHIMA†

Wildlife and Ecology Unit, Veterinary Genetics Laboratory, School of Veterinary Medicine, and †Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616, USA*Abstract**

We developed 10 polymorphic microsatellite loci for Yellow-billed Magpies (*Pica nuttalli*). The primers were tested across a population of 57 Central California Yellow-billed Magpies and displayed an average of 3.9 alleles per locus. Forty-one American Crows (*Corvus brachyrhynchos*) from California were polymorphic for seven of the loci with an average of 2.9 alleles per locus. One additional microsatellite-containing locus displayed diagnostic allele sizes and may be useful to distinguish between the two species. These corvid specific microsatellites will aid ecological studies of the population-level effects of diseases, such as West Nile virus.

Keywords: *Corvus brachyrhynchos*, crow, magpie, microsatellite, *Pica nuttalli*, West Nile virus

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West Nile virus, habitat loss, and mortalities due to agricultural pest control endanger the population viability of the yellow-billed magpie (*Pica nuttalli*), a corvid species (Family Corvidae) with a range limited to Central California (Reynolds 1995). Numerous indicators suggest that tens of thousands of yellow-billed magpies died following the entrance of West Nile virus into their habitat in 2004 (S. P. Crosbie *et al.* in review); however, little is understood of their population biology, and their post-West Nile census size is unknown. In order to assess and compare the genetic diversity and population structure before and after the West Nile virus epidemic struck the species, we developed microsatellite markers specifically for yellow-billed magpies. In addition, we screened the markers with samples from American Crows (*Corvus brachyrhynchos*) to assist studies of the impact of the virus on this species.

Four libraries enriched with microsatellite motifs (AAAC₈, AAAG₈, AAAT₈, and GAGAA₇) were created by Genetic Identification Services with DNA extracted from a muscle sample collected from a yellow-billed magpie following

the methods of Jones *et al.* (2002) and as detailed in Hull *et al.* 2007. Of 228 clones sequenced, 136 contained a microsatellite. We used PRIMER 3 version 0.2 (Rozen & Skaletsky 2000) and MREPS version 2.5 (Kolpakov *et al.* 2003) to design 42 primers with melting temperatures of 60 °C. QIAGEN DNeasy kits (QIAGEN) were used to extract DNA from muscle samples collected from dead birds submitted to the West Nile virus surveillance programme conducted by the California Department of Health Services (Reisen *et al.* 2004). Of 42 loci initially screened in DNA from 10 yellow-billed magpies, 11 displayed at least two alleles, no more than two alleles per individual and amplified products of expected size. These loci were fluorescently dye-labelled, combined into three multiplex groups (Table 1), and screened in DNA from 57 yellow-billed magpies from one population (Sacramento and Yolo counties, California) and 41 American crows sampled across California.

Multiplex polymerase chain reaction (PCR) was carried out in 12.5 µL reactions containing 1× PCR buffer II, 10 mM Tris-HCl pH 8.3 (at 25 °C), 50 mM KCl (ABgene), 2.5 mM MgCl₂, 0.2 mM dNTP (Fisher Scientific), 0.5 U of ThermoPrime Plus DNA Polymerase (ABgene), 1 µL DNA (20–60 ng) and various concentrations (Table 1) of forward fluorescent dye-labelled (Applied Biosystems Inc.) and

Correspondence: H. B. Ernest, Fax: 530-754-5518; E-mail: hbernest@ucdavis.edu

Table 1 Characterization of 10 polymorphic microsatellite loci developed for yellow-billed magpies (YBMA), *Pica nuttalli* and also tested in American crows (AMCR) (*Corvus brachyrhynchos*). M, multiplex group identification number; A, number of alleles; H_O , observed heterozygosity; H_E , expected heterozygosity; Null, estimated frequency of null alleles using CERVUS 3.0 (Kalinowski *et al.*, 2007; note that strongly positive values are indicative of null alleles); F, forward primer; R, reverse primer. *Primers lacking in polymorphism or heterozygosity in crows and therefore not included in analysis. †Repeat motif for PnuA408w is (AAACC)₆(AACCC)₃(CAA)₄(CAAAACCAAA)₂. All 57 yellow-billed magpies and 41 American crows that were screened produced genotypes except: PnuA3w failed to produce genotypes for two yellow-billed magpies, and one crow each failed to amplify at Pnu5A5Gw, PnuA408w, PnuC107w, and PnuC405w (the last two loci failed to generate a genotype for the same individual crow)

Locus	Dye label	PCR product size	Repeat motif	A		H_O YBMA	H_F YBMA	Null YBMA	A		H_O AMCR	GenBank Accession no.	Primer (μM)	Primer sequence
				M	YBMA				YBMA	AMCR				
PnuA106w	PET	160–176	(TGTT) ₁₁	1	5	0.737	0.72	-0.02	4	0.41	EF580123	0.05	F: GTATTTTGGGATGTCITTAGGGTTG R: CACACTCGAGCTACAATAAACCTG	
PnuA120w	PET	220–236	(AAAC) ₉	3	3	0.579	0.619	+0.03	1	*	EF580124	0.05	F: AAATACTGAAATAGCCACCAGGTC R: GGCTCAGATACCTATTGAAATATGAC	
PnuA2w	NED	185–197	(AAAC) ₁₁	1	3	0.158	0.149	-0.03	4	0.073	EF580121	0.075	F: TGCCAGTGCACCTCATTTACTT R: TGTCTTTATTTTAGGCTTTGC	
PnuA3w	NED	254–260	(TTTG) ₁₂	3	3	0.421	0.492	+0.05	5	0.366	EF580122	0.05	F: GACAGGAGCCCACTTTCTG R: GGCTTCCAAAGGTAGTCTTC	
PnuA408w	6FAM	226–242	†	2	5	0.737	0.72	-0.02	3	0.35	EF580125	0.075	F: ATAACTGTCAGACTGTCRAGA R: TTGTCCACGAGTGCAGGAAATGTAT	
PnuB213w	PET	136–148	(AAGA) ₈	2	4	0.474	0.469	+0.01	1	*	EF580126	0.075	F: GCAGCTTTGTAGAGGACTTGT R: TTTGAAATGTCCTCCAGTTTC	
PnuC107w	VIC	149–157	(AAAT) ₈	1	3	0.368	0.532	+0.18	3	0.175	EF580127	0.03	F: CCAAGCCTACACTACTCCAATTC R: ACAGTCTGCTAGGTTTCAFCAGC	
PnuC222w	PET	161–185	(TAAA) ₈	1	4	0.333	0.363	+0.03	2	0.21	EF580128	0.075	F: CCAAGCCTACACTACTCCAATTC R: CATGCCAAATCTTTGGTAAAGACA	
PnuC405w	PET	101–121	(TAAT) ₉	1	4	0.649	0.657	+0.00	1	*	EF580139	0.075	F: TTGCGTTCAFCAGGGAAGG R: ATTGGAGTTTGCCTGTCAATCA	
PnuC424	NED	146–178	(AAAT) ₈	3	5	0.754	0.648	-0.09	2	0.561	EF580131	0.05	F: CCCTGGCTCCTGTGACTAAAGTA R: GCCCAATTAATTTGTATGAGGAAA	

reverse primers (BioSource) ranging from 0.05 to 0.15 μM . Thermal cycling parameters were: 94 °C for 1 min followed by 30 cycles of 94 °C for 45 s, 60 °C for 30 s and 72 °C for 30 s and a final 30-min extension at 72 °C followed by 15 °C until further use. PCR products were separated with a 3730 DNA Analyser (Applied Biosystems) with each capillary containing 1 μL of a 1:11 dilution of PCR product and deionized water, 0.05 μL GENESCAN 500 Liz Size Standard and 9.95 μL of HiDi formamide (both products Applied Biosystems) that was denatured at 95 °C for 3 min. Products were visualized with STRAND version 2.3.79 (Toonen & Hughes 2001).

Ten loci were polymorphic in the magpies (number of alleles per locus ranged from three to five with an average of 3.9), while seven loci were variable in the crows (alleles per locus ranged from two to five with an average of 2.9). In magpies, expected heterozygosity (H_E) ranged from 0.15 to 0.72 while the observed (H_O) was from 0.16 to 0.75. In crows, H_E ranged from 0.14 to 0.51 while the H_O was from 0.07 to 0.56 (GENEPOP version 3.4, Raymond & Rousset 1995) (Table 1). One additional microsatellite-containing locus (PnuD110w) displayed two bands at 121 bp and 127 bp for all magpies, while all crows displayed only one band at 117 bp, thus providing a potentially useful species discrimination tool (primer sequences: F: GTAGCGATCGTAGCACTCGAC; R: TTTGGGACACCAGAACCAC; repeat motif (AAAGAG)₃₀; label VIC; multiplex no. 2). None of the polymorphic loci deviated from Hardy–Weinberg and linkage equilibrium expectations (GENEPOP) in magpies; however, one locus displayed a high estimated frequency of null alleles (Table 1; locus PnuC107w; CERVUS version 3.0; Kalinowski *et al.* 2007). In crows, PnuA2w and PnuC107w deviated from Hardy–Weinberg equilibrium and the locus pair of PnuA106w and PnuA408w displayed significant linkage disequilibrium, perhaps in part due to sampling from multiple regions of California. None of the primers displayed significant overlap with previously published corvid sequences. These primers have the potential to facilitate the ecological knowledge and contribute to population health surveillance of the yellow-billed magpie and other corvid species.

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