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PRIMER NOTE

Characterization of polymorphic microsatellite loci for the invasive monk parakeet (*Myiopsitta monachus*)

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Abstract

Microsatellite loci were characterized for the monk parakeet (*Myiopsitta monachus*) from a GT_n-enriched genomic library. Twelve of 14 microsatellite loci were polymorphic, averaging 6.7 alleles per locus across the 20 individuals genotyped. Mean expected heterozygosity was 0.72, with locus-specific values ranging from 0.53 to 0.90. An equally high multilocus probability of identity (2.48×10^{-12}) was revealed for this set of loci. In addition, all 12 loci were demonstrated to cross-amplify to varying extents within three additional parrot genera suggesting their potential utility for population-level studies in a broad range of Neotropical psittacines.

Keywords: dinucleotide, invasive species, microsatellites, polymorphic, primers, Psittacidae

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Monk parakeets (*Myiopsitta monachus*) are among the most widely successful parrot invaders. Native to the lowlands of southern South America, *M. monachus* has established self-sustaining breeding populations in such disparate regions as Puerto Rico, Kenya, Japan, Europe and throughout the United States, most notably in Florida and Connecticut. In general, US naturalized populations are a collection of disjunct colonies, most common in southern and coastal regions, with an estimated 6000–200 000 individuals in residence nationally (Van Bael & Pruett-Jones 1996). A notorious crop pest across its native range, *M. monachus* has not had a measurable impact on US agriculture to date, although they remain a persistent threat because of an estimated population doubling every 5.4 years (Pruett-Jones & Tarvin 1998). A less publicized, but economically significant impact of the monk parakeet invasion has been their preference for power structures as nesting substrates. In support of a larger study of the behavioural, cultural and genetic mechanisms of monk parakeet invasion success, we report here the characterization of 12 polymorphic microsatellite loci for *M. monachus*.

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Enriched genomic library construction and microsatellite isolation followed Hamilton *et al*. (1999) as described in Russello *et al*. (2001) using genomic DNA isolated from two females sampled in Entre Rios, Argentina. Fourteen primer pairs flanking microsatellite regions of ≥ 7 dinucleotide repeats were designed from 48 sequenced clones using PRIMER 3 software (Rozen & Skaletsky 1998).

Blood samples collected from 20 *M. monachus* individuals in Entre Rios, Argentina were used to assess polymorphism of the isolated markers. DNA was extracted using the QIAGEN DNeasy kit based on manufacturer's protocols for isolation of genomic DNA from whole nucleated animal blood. Polymerase chain reactions (PCR) were performed using an MJ Research DNA Engine Thermal Cycler and carried out in a 12.5-μL volume containing: ~20–50 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.5 mM $MgCl_2$, 200 μM dNTPs, 7.5 μg bovine serum albumin (BSA), 0.8 μM of each primer and 0.5 U of Ampli*Taq* Gold DNA polymerase (PE Biosystems). Reaction conditions for all primers employed a 'touchdown' cycling program consisting of: 95 °C for 10 min; 35 cycles at 95 °C for 30 s, annealing for 30 s, and 72 °C for 45 s; and a final step at 72 °C for 7 min. The annealing step in the touchdown program decreased by 2 °C every other cycle from 59 °C until it reached 51 °C

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Table 1 Polymorphic dinucleotide microsatellites characterized for *Myiopsitta monachus*

Locus	Primer sequences 5′–3′	Repeat structure	T_a (°C)	No. of alleles	Product size ranges (bp)	H_O	H_E	P_{ID}	Accession no.
MmGT012	F: GGCACGAATCTAGCATTTCC R: TCAGTGACGGAGACTTGCTG	$(CA)_7GA(CA)_2$	59 → 51	7	320–340	0.80	0.77	0.10	EF405606
MmGT030	F: GATCCCCACCTTCAGATTCA R: AATGCGACAGTTAGCACTTGA	$(CA)_{17}TA(CA)_7$	59 → 51	10	316–336	0.90	0.85	0.05	EF405607
MmGT046	F: ACACAGCCATCCGGATACA R: TGTTACTGGGGTAAGAAGATACCA	$(CA)_8CG(CA)_2$	59 → 51	4	178–186	0.65	0.53	0.28	EF405608
MmGT054	F: TGGGTTTACAAAAGGCCAAA R: CAAGCTCAGACACAGGGATG	$(GT)_{12}$	59 → 51	6	162–182	0.26	0.59	0.21	EF405609
MmGT057	F: TCACTGAGCCTTGAACACATCT R: ACCAGACCCGCCTTTTGTAT	$(CA)_8AA(CA)_7$	59 → 51	8	131–147	0.65	0.79	0.08	EF405610
MmGT060	F: TGTGAGATTTAGTCTTGCTGGA R: CCATATTGTAAGTCTGGAGACATGAT	$(GT)_9TT(GT)_3$	59 → 51	5	108–124	0.60	0.59	0.25	EF405611
MmGT071	F: AATATAACTGGTGATAGCATGAAGC R: GTGTTGCAGTCATTGCTGGT	$(GT)_9$	59 → 51	6	220–234	0.60	0.77	0.10	EF405612
MmGT090	F: AGCTCAGCTCCTGAAAAATGA R: CTCATCAGCAGCAACACGAG	$(GT)_{10}$	59 → 51	4	229–239	0.65	0.62	0.12	EF405613
MmGT098	F: CAGCACAAAGGAAACCCATT R: GGCCCAAGTTCATTACTTACG	$(CA)_{21}$	59 → 51	8	168–194	0.53	0.78	0.09	EF405614
MmGT105	F: TGGCAAGACCCCTTTGTC R: TGTCTCTGTTTTCCCATGC	$(CA)_{12}CG(CA)_7$	59 → 51	7	213–237	0.79	0.72	0.12	EF405615
MmGT148	F: TTGCTGCAGTATGTGAGAAGTGTTG R: TTCTCTTCCCTCTGCACTCATTACAT	$(GT)_{13}$	59 → 51	11	273–307	0.95	0.89	0.02	EF405616
MmGT160	F: AGAGATGGCAAAAAGGAAAAACCCTAATA R: GGTCTTGGCAGCAGCGCTGTA	$(GT)_7$	59 → 51	4	150–158	0.58	0.66	0.18	EF405617

T_a is the annealing temperature used in 'touchdown' PCRs. Number of alleles, product size ranges, proportion of observed (H_O) and expected heterozygosities (H_E), and probability of identity (P_{ID}) are all based on the genotyping of 20 individuals of *Myiopsitta monachus*. GenBank Accession nos are also indicated.

(the 9th cycle) at which point the remaining cycles continued with a 51 °C annealing temperature.

All forward primers were 5′-tailed with an M13 sequence (5′-TCCCAGTCACGACGT-3′) to facilitate automated genotyping. Specifically, the M13-labelled forward primer was used in combination with an M13 primer of the same sequence but 5′-labelled with one of three fluorescent dyes (6-FAM, NED, VIC). In this manner, the fluorescent label was incorporated into the resulting PCR amplicon (Boutin-Ganache *et al*. 2001). In addition, a 5′-pigtail was added to all reverse primers to reduce stutter and improve the reliability of allele calls according to the procedure outlined in Brownstein *et al*. (1996). All genotypes were collected on an ABI PRISM 3730 and analysed using GENEMAPPER 3.1 (Applied Biosystems).

Allelic diversity, observed and expected heterozygosities (H_E) were calculated for each locus. Tests for deviation from Hardy–Weinberg equilibrium and linkage disequilibrium were conducted, as implemented in GENEPOP (Raymond & Rousset 1995), and corrected for multiple comparisons using the sequential Bonferroni procedure (Rice 1989). The probability of identity (P_{ID}), or the probability of obtaining identical genotypes given an allele frequency distribution, was calculated for each locus and across all loci as a measure of the power of the developed microsatellites to resolve between different individuals (Paetkau & Strobeck 1994).

Twelve of 14 tested loci were polymorphic, reliably amplifying alleles consistent with a stepwise mutation model. The number of alleles per locus varied from four to 11 and averaged 6.7 across all loci (Table 1). Mean H_E was 0.72 with locus-specific values ranging from 0.53 (MmGT046) to 0.90 (MmGT148; Table 1). Significant deviation from Hardy–Weinberg equilibrium was recorded at only a single locus (MmGT054) following sequential Bonferroni correction. In this case, departure from Hardy–Weinberg expectations was due to a significant excess of homozygotes. Furthermore, there was no evidence of nonrandom association of genotypes in any of the pairwise tests for linkage disequilibrium performed for all possible pairwise comparisons of the sampled loci. Probabilities of identity for each locus are listed in Table 1. The most powerful locus for resolving between individuals was MmGT148 (P_{ID} = 0.021) with MmGT046 exhibiting the lowest probability of identity (P_{ID} = 0.28). Applying the product rule, the probability of randomly obtaining identical multilocus

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Table 2 Cross-species amplification of *Myiopsitta* microsatellite loci within single individuals of five additional Neotropical parrot taxa

Locus	Blue and Gold Macaw *Ara ararauna*	Green-winged Macaw *Ara chloroptera*	Orange-fronted Conure *Aratinga canicularis*	Yellow-naped Amazon *Amazona auropalliata*	St. Vincent Amazon *Amazona guildingii*
MmGT012	–	–	–	–	–
MmGT030	–	–	–	–	–
MmGT046	+	–	+	+	+
MmGT054	+	+	+	+	–
MmGT057	–	–	+	+	–
MmGT060	–	–	+	+	+
MmGT071	–	–	–	–	–
MmGT090	+	+	+	+	+
MmGT098	–	–	–	–	–
MmGT105	+	+	+	+	+
MmGT148	+	+	+	+	+
MmGT160	–	–	+	+	+

Successful cross-species amplification (with reaction profiles optimized for *Myiopsitta monachus*) denoted by +, unsuccessful denoted by –.

genotypes for this set of loci was 2.48×10^{-12}. Genotyping of two breeding pairs and six of their known offspring confirmed Mendelian inheritance of all markers (data not shown).

Cross-species testing was performed for all loci in five additional species representing three Neotropical genera (Table 2). Cross-species amplification was successful for at least three loci for all taxa. Moreover, five loci were found to amplify across all genera tested demonstrating the potential utility of these markers for population-level studies of a broad range of Neotropical parrot taxa.

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