TECHNICAL NOTE

Characterization of 10 polymorphic microsatellite markers for the purple martin, *Progne subis*

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Abstract The purple martin is a large North American swallow that suffered a severe population crash in the 20th century due to nesting competition from invasive species. We screened an enriched DNA library for microsatellites from purple martins (*Progne subis subis*). Ten loci consistently amplified and were polymorphic with an allele range of 2–19 and observed heterozygosity ranged from 0.31 to 0.93. Two loci are sex-linked, and two additional loci failed to meet Hardy–Weinberg expectations likely due to the presence of null alleles. These polymorphic microsatellite loci can be used for studies of genetic diversity, population structure, and relatedness, all of which have been suggested to determine the impact of substantial regional declines in population density.

Keywords Purple martin · *Progne subis* · Microsatellite · Genetic marker · Genetic variation

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Purple martins (*Progne subis*) are the largest migratory avian species in the swallow family, Hirundinidae and are exclusively colonial cavity nesters (Doughty and Fergus 2002). Both eastern (*P.s. subis*) and western populations (*P.s arboricola*) of purple martins have been in decline since the mid-20th century (Laughlin and Kibbe 1985; Zeransksi and Baptist 1990; Veit and Petersen 1993; Hunt 2003). They have suffered severe range-wide declines associated with the loss of territory due to nesting site competition with european starlings (*Stutnus vulgaris*) and house sparrows (*Passer domesticus*; Brown 1981; Baker et al. 2008). Data from the Breeding Bird Survey indicates a range decline of 0.6% per year, with the decline concentrated in eastern North America (0.9%; Sauer et al. 2004). Even though the species as a whole does not have IUCN status, certain populations have been Red-listed as late as 2006, and are currently Blue-listed or vulnerable (Baker et al. 2008). Purple martins are particularly vulnerable to population decline because they are dependent on humans to provide nesting cavities (Copley et al. 1999). Microsatellites characterized in this study can be used to assess levels of genetic variation existing in current populations. Highly variable microsatellite markers are excellent genetic markers for population-wide studies of genetic diversity, relatedness, paternity assignment, and testing population genetics hypotheses. We report the isolation and characterization of 10 polymorphic microsatellite loci from purple martins (*P. subis subis*) and characterization in a population from Front Royal, Virginia.

Blood samples were collected in 1993 as described in Wagner et al. (1996). DNA was extracted with phenol–chloroform-isoamyl alcohol and quantified using a Nano-Drop® spectrophotometer. Microsatellites were isolated as described by Glenn and Schable (2005). HaeIII and RSAI fragments between 200 and 500 bp from whole genomic

DNA were ligated double-stranded linkers (Forward 5′ GTTT TAA GGC CTA AGC AGA ATC 3′ and reverse 5′ pGAT TCT GCT AGC TAG GCC TTA AAC AAAA 3′) using USB Ligate-IT and hybridized to two different biotinylated oligonucleotide tandem repeat probes: Mix 1, (AG)₁₂, (TG)₁₂, (AAC)₆, (AAG)₈, (AAT)₁₂, (ACT)₁₂, (ATC)₈, Mix 2, (AAAC)₆, (AAAG)₆, (AATC)₆, (AATG)₆, (ACAG)₆, (ACCT)₆, ACTG)₆. Amplification of hybridized products was performed using the following thermalcycling conditions: 95°C, 5 min, 70°C, step down 0.2°C/5 s until 50°C, 10 min, step down 0.5°C every 5 s until 40°C, 4°C hold. Dynabeads were captured with a magnet leaving behind supernatant that contained only fragments with microsatellites. Fragments were cloned using a Topo-TA Cloning® kit (Invitrogen Corp.). Plasmid DNA was isolated and purified using a Wizard® Plus Miniprep DNA purification kit (Promega Corp.) and plasmid inserts were sequenced on a LiCor 4300 DNA analyzer. Sequence-specific primers were designed using Primer3 (Untergasser et al. 2007).

We identified and sequenced 200 clones of which 29 were acceptable for primer design and amplification. Genotyping was performed on a LiCor 4300 automated DNA analyzer in 10 μl reactions for 25 presumably unrelated adult birds. Ten loci showed clear genotypes and amplified consistently (Table 1). Genotypes were scored by reference to 4–8 size standards/gel using Gene ImagIR™ software.

The 10 microsatellite loci were highly polymorphic (Table 1) with a mean observed heterozygosity of 0.45 [range = 0.31–0.91], a mean number of alleles of 7.60 [range = 2–19]. PUMA14 and PUMA91 showed no heterozygous bands in females. If we assume the markers are in Hardy–Weinberg equilibrium (HWE), the probability of observing no heterozygous females in our sample (n = 8 females) is 3.9×10^{-3} for PUMA14, and 1.4×10^{-3} for PUMA91. It is highly likely that these loci are sex-linked. Two loci (PUMA49 and PUMA74) deviated significantly from HWE in the direction of heterozygote deficiency using an exact test and MCMC simulation to calculate probabilities (Weir 1990) as implemented in Powermarker (Liu and Muse 2005). The heterozygote deficiency is likely due to null alleles originating from polymorphic SNPs in the primer sites. There was no significant linkage disequilibrium between any loci.

We calculated individual paternity exclusion probabilities for each locus (Table 1) using CERVUS (Kalinowski et al. 2007). The average probability across loci of not

Table 1 Microsatellite loci isolated from the purple martin (*Progne subis subis*) and measures of genetic variation, tests for Hardy–Weinberg equilibrium, and estimates of exclusion probability for paternity analysis

Marker	Repeat motif	Primer sequence (5′–3′)	N	Size Range (bp)	Na	Ho	He	PIC	Ex
PUMA 14	(GAAA)₂₇	F: TGAGAGAAAGAGAAGGAAAGAAGG	20	278–346	15	0.91	0.50*	0.90	0.32
		R: GAATCAGAGAAAATAACATTTGAGG							
PUMA 19	(AAAC)₄	F: ACTATGTCATTCACCTTCAAGTGG	22	224–228	2	0.47	0.41	0.36	0.89
		R: CTCTTCTCTGCCTCAGGAAACC							
PUMA 49	(CAAA)₉	F: AAAACCACAAACAAACACACAAAA	24	220–252	7	0.66	0.38*	0.63	0.73
		R: GAAAGAACTTCAAATTCAGGGAAA							
PUMA 66	(CCTTCT)₂₅	F: TCATTTATGATCAAGGATTCACC	21	328–440	19	0.90	0.94	0.91	0.28
		R: TGAGTTCATGTAAGCAAGTTCACC							
PUMA 74	(GAAGA)₁₉,	F: CAATTATCTATCCCACCTCTTTGC	23	404–490	19	0.91	0.26*	0.93	0.24
	(GAAAA)₂₄	R: ACAGAAAATATTGGTTTGCTCTCC							
PUMA 87	(CA)₅	F: TTCTGTTTGATCCCTAATCTAATGG	25	232–236	4	0.31	0.28	0.31	0.94
		R: AACACAAGACTTTCAACATTCTTCC							
PUMA 91	(GAAA)₃₁	F: TGGTTGGCAATGATTAAGGAC	22	232–336	20	0.93	0.59*	0.92	0.25
		R: GACCCTGGTGTGTATTATTCAATC							
PUMA 98	(GTTT)₅	F: TCTCCTCTCTCTTTCTCTCTCTCG	23	180–204	9	0.87	0.83	0.85	0.45
		R: TACATATGGATTCATGGATTGACC							
PUMA 133	(AAAC)₅	F: CTGCAGCCAAAAAGATCAGC	24	242–246	3	0.50	0.33	0.41	0.87
		R: TTTGCCATAATGCTGTTACG							
PUMA 159	(TC)₆	F: CAAGCTTTCCCCTTATCAGC	23	196–200	3	0.52	0.65	0.42	0.87
		R: GGGGAGGAGTGAGAGAGAGC							

The number of individuals genotyped (*N*), Size range in base pairs (bp) of PCR products, number of alleles (*Na*), observed Heterozygosity (*Ho*), expected heterozygosity (*He*), polymorphic information content (*PIC*), probability of not excluding a candidate father (Ex). *statistically significant deviation from Hardy–Weinberg equilibrium (*P* < 0.01)

excluding a candidate parent of an arbitrary offspring given that the genotypes of one parent are known, as is typically the case with purple martins because they can be captured in the evening while the mother is in the nest with the chicks, is 4×10^{-5} and 2×10^{-2} excluding the loci that deviate from HWE. We have confirmed the use of these loci for paternity analysis using three of the loci (PUMA19, PUMA49, and PUMA98; Stanley 2008) by comparison to paternity analysis from a previous study that used multilocus VNTR markers in the 1993 sample (Wagner et al. 1996). Segregation of bands in each of these cases was consistent with the previous VNTR paternity analysis. The microsatellite markers we have identified here are thus useful for studies of genetic variation, population structure, and mating strategies.

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