Conservation Genetics **5:** 861–864, 2004.

Technical Note

Development of polymorphic microsatellite loci in the northern goshawk (*Accipiter gentilis*) and cross-amplification in other raptor species

J. Rick Topinka* & Bernie May

*University of California, Davis, Department of Animal Science, Meyer Hall, One Shields Ave, Davis, CA 95616, USA (*Author for correspondence: Phone: (530) 752-6351; fax: (530) 752-0175; e-mail: jrtopinka@ucdavis.edu)*

Received 11 December 2003; accepted 3 March 2004

Key words: Accipiter gentilis, genetics, microsatellite, northern goshawk, raptor

The northern goshawk (*Accipiter gentilis*) is the largest species in the genus *Accipiter*, a widespread group of hawks that feed on small birds and mammals. In North America there are three proposed subspecies: the Queen Charlotte goshawk, *A. g. laingi*, from the coastal islands of southeastern Alaska, the Apache goshawk, *A. g. apache* which occupies the sky island forests of the southwestern US and Mexico, and *A. g. atricapillus,* the most widespread subspecies, which is found throughout the rest of the species' North American range. The discrimination of subspecies can be a difficult and contentious issue. In the case of the northern goshawk, the original descriptions of the three North American subspecies have not yet been corroborated by either rigorous genetic or morphometric analysis. We have developed this suite of microsatellite loci to investigate the population structuring of the northern goshawk as well as to assist in the determination of subspecific status for the northern goshawk in North America.

We combined DNA from two different species, Kearney's bluestar (*Amsonia kearneyana*) and the northern goshawk (*Accipiter gentilis atricapillus*) prior to beginning the library construction process in order to maximize cost and time efficiency. As the development expenses for microsatellite libraries are often cost prohibitive for a single species, we were interested in determining the feasibility of isolating sufficient numbers of microsatellites from each of the species included in multi-species DNA libraries. Repeat motifs occur at different frequencies in different taxa, so we used enrichment procedures for four distinct repeat motifs in order to create four composite libraries each intended to be preferentially enriched for one of the two species (Toth et al. 2000). In constructing this composite library we used a plant and a bird species, as both are believed to have relatively low, and possibly equivalent numbers of microsatellites (Lagercrantz et al. 1993; Primmer et al. 1997).

We purified genomic DNA from leaf samples of Kearney's bluestar, an endangered perennial plant from Arizona, using the CTAB method (Doyle and Doyle 1987) followed by phenol/chloroform extraction and ethanol precipitation, and from northern goshawk blood using Qiagen Dneasy™ Kits, and then combined the purified DNA in equal proportions. Libraries enriched for $(GA)_n$, $(TACA)_n$, $(TAGA)_n$ and $(AAT)_n$ repeat motifs were constructed by Genetic Identification Services (Chatham, CA) and screened according to the protocol in Meredith and May (2002). Briefly, libraries were constructed with partial digests of genomic DNA and ligation of a *Hin*dIII restriction site-oligonucleotide linker, and enriched for the four repeat motifs by magnetic bead capture. Five hundred and seventy two plasmids were purified using the Qiagen Qiaprep® Kit and cycle sequenced using Applied Biosystems BigDye™ Terminator Cycle Sequencing Ready Reaction kit version 1.0 and pUC19 forward or reverse

Table 1. Primer sequences for *Accipiter gentilis* microsatellite loci

Locus	Repeat motif	Primer sequence (5′–3′)	Clone size (bp)	GenBank accession no.	*n*	Number of alleles	Allele size range (bp)	H_o	H_E
Age1	$(GGGAA)_8GA(GAGAA)_9$ $GAGAG(GAGAA)_3$	**F**: ATAGGGATTGGGACATTTTAG **R**: TTTAGGAACCGCAAGACTG	216	AY312451	7	7	196–250	1.00	0.88
Age2	$(GAGAA)_{10}(GA)_4$	**F**: CTGATGTATATTTGGGTGCTGGAG **R**: CTGGCTGCGGCTTGTGTC	170	AY312452	10	7	158–198	0.90	0.85
Age3[a]	$(TATC)_4..(TATC)_3..(TA)_3$ $TC(TA)_5$	**F**: GTCAGGGCAAATAAAAATAAAACA **R**: AGACAAACTGAAACAATGCCTAAG	166	AY312453	7	1	166	0.00	0.00
Age4	$(GAGAA)_{19}$	**F**: CAGCGTGTTTTCAGGGAGCAG **R**: GACAGGGACGTGAGGATTGATTCT	275	AY312454	12	7	235–275	0.83	0.83
Age5	$(AAT)_{10}$	**F**: ACGTTACAGACACCGATTACTTCC **R**: AGCCACGCGTCTGATACTTT	158	AY312455	11	3	149–158	0.55	0.56
Age6	$(GAGAA)_2GA(GAGAA)_2$ $GAA(GAGAA)_5$	**F**: GAAATGCCAGAGTTGAGTATGATA **R**: CATTGCCCAGCCTGAAA	259	AY312456	10	5	310–390	0.20*	0.76
Age7	$(GA)_{27}$	**F**: GGGGCATTGTGCTATTAGAAGTGA **R**: GGAGGCCCCCAGACAAAAG	249	AY312457	11	5	225–245	0.64	0.67
Age8	$(CAT)_{11}$	**F**: CACGGCTTATTTAACCACCCTATT **R**: TGCCCACTTTTCTTCTTCCATC	133	AY312458	12	3	127–139	0.60	0.62
Age9	$(CT)_{13}$	**F**: TCCTGTACAGAAAAGAAAAGAGAT **R**: CTGAGCAGCCTGAAACCTA	190	AY312459	10	4	184–194	0.70	0.69
Age10	$(GA)_2(CA)_3(GA)_{18}$	**F**: ATACCCTCATGCTCCTGTTCC **R**: TCCTGGGCTGATAGTGGTC	163	AY312460	6	4	151–159	0.33*	0.77
Age11	$(AAT)_{12}$	**F**: GTTATTTCAGGTGGGGCTCATTCA **R**: GCTACGGCGGCTGCTAAGTCA	240	AY312461	9	4	237–249	0.40	0.49
Age12[ab]	$(GAGAA)_5(GAGAGAA)_5..$ $(GAGAGAA)_4..(GAGA-$ $GAA)_6(GAGAA)_{12}$	**F**: GCAAGCTCCGTGCTAGAG **R**: ACTTCAACCTAAACTTTCCACA	412	AY312462	–	–	412	–	–

n, number of individuals analyzed; H_O, observed heterozygosity; H_E, expected heterozygosity; **F**, forward primer; **R**, reverse primer; interrupted microsatellites are indicates by a (..) between motifs; all loci were amplified using the same touchdown PCR protocol; [a]polymorphic in other raptor species; [b]poor amplification in *A. gentilis*, *significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$). Allele size ranges were estimated from molecular size standards on gels.

sequencing primers. We sequenced 211 clones from the $(GA)_n$ library, 187 from the $(AAT)_n$ library, and 87 from each of the $(TAGA)_n$ and $(TACA)_n$ libraries. Cycle sequencing reactions were purified using RapXtract™ magnetic bead separation (Prolinx®), combined 2:1 with formamide loading buffer and denatured at 65 °C for 3 min. Samples were then separated on an MJ Research BaseStation™ using standard sequencing protocols. We assembled sequences using SeqMan™ software and designed PCR primer pairs for 46 distinct microsatellite repeats using PrimerSelect™ (Lasergene 4.0, DNASTAR Inc.). When possible, we designed the primer pairs to amplify fragments that ranged from 150–250 bp.

As the libraries were composites of two distinct species DNA, we initially screened primer pairs against four individuals of each species to determine which species the clone was derived from. We used 5 ng of genomic DNA as a template in a 10 μl reaction with the following conditions: 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 0.1% Triton® X-100; 1.5 mM $MgCl_2$; 0.2 mM each dNTP; 1 mM each unlabelled forward and reverse primers; and 0.375 units Taq polymerase (Promega). We used a touchdown protocol with the following parameters for PCR amplification: 95 °C for 1 min followed by 30 cycles of 95 °C for 1 min, 67 °C for 45 s with 0.5 °C decrease each cycle and 72 °C for 2 min. Amplification reactions were combined 1:1 with 98% formamide loading buffer (10 mM EDTA pH 8.0; 0.005% xylene cyanol and 0.005% bromophenol blue) denatured at 95 °C for 3 min and chilled on ice prior to loading. Samples were electrophoresed on a 5% denaturing polyacrylamide gel at 50 W for 70 min. Gels were stained using a SYBR® Green-agarose overlay protocol and scanned on a Molecular Dynamics™ FluorImager 595 (Rodzen et al. 1998).

Nineteen primer pairs of the 46 developed amplified appropriately sized fragments in northern goshawk and 18 amplified in Kearney's bluestar (manuscript in preparation). We report twelve goshawk microsatellite loci here, the remaining seven either did not amplify well or amplified many nonspecific bands and further optimization efforts did not result in strong, specific amplification. Primer pairs that amplified a fragment of the expected size in the four initial goshawk samples were then screened against 12 adults from a population on the Kaibab Plateau in northern Arizona. Expected and observed heterozygosities are reported from up to 12 samples from this single population (Table 1). We tested for Hardy-Weinberg equilibrium (HWE) with the software package GENPOP 3.3 using exact tests (Yeh and Boyle 1997). Two loci, Age-6 and Age-10 were found to be significantly out of HWE ($P < 0.05$). This may be due to the presence of null alleles at these loci and will require further analysis. We used GENEPOP 3.3 to conduct exact tests for linkage disequilibrium and found no significant values ($P < 0.05$).

In contrast to previous experience in this laboratory, many of the repeat motifs in the isolated microsatellites do not exactly match those used in the enrichment protocol. This result may be because the specific repeat motifs used for enrichment are under-represented in the genomes of these taxa. The $(GA)_n$ enriched library yielded many clones with $(GAGAA)_n$ based repeats for

Table 2. Cross-species amplification of *Accipiter gentilis* microsatellites

Species	Locus											
	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
Accipiter striatus	–	–	1	–	2	1	2	2	1	±	1	±
A. cooperii	±	4	2	4	2	2	3	3	2	2	2	±
Haliaeetus leucocephalus	–	±	1	±	2	1	1	–	1	–	3	3
Buteo jamaicensis	3	3	1	±	3	3	1	±	–	–	2	3
B. swainsoni	±	4	2	3	4	±	1	±	1	±	1	2
Falco sparverius	–	–	–	–	2	–	1	–	–	–	–	–

Two individuals of each species were screened: n, number of alleles; ± weak amplification; – no amplification.

the northern goshawk, that the $(GA)_n$ enrichment scheme would be expected to select for as well.

We screened all primer pairs that successfully amplified against two individuals from each of a suite of additional raptor species, regardless of whether they were monomorphic or polymorphic in northern goshawk (Table 2). We used identical PCR reaction conditions and touchdown thermocycler programs to those described above. Most of the primers described here cross-amplify other *Accipiter* as well as *Buteo* species, and a number cross-amplify in the genera *Haliaeetus* and *Falco*. These markers should prove useful to researchers studying a variety of raptor species.

Acknowledgements

Support for this work came from a UC Davis Block Grant Fellowship and National Science Foundation, Graduate Research Fellowship (JRT). We thank Richard Reynolds (Colorado State University and USDA Forest Service), Tom Gavin (Cornell University), Mary Delany (UC Davis) and Holly Ernest (UC Davis) for providing blood or DNA samples used in developing this library.

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