

PRIMER NOTE

DNA microsatellites of pronghorn (*Antilocapra americana*)

M. D. CARLING, C. W. PASSAVANT and J. A. BYERS

*Department of Biological Sciences, University of Idaho, Moscow, ID 83844-3051, USA***Abstract**

We developed a set of eight polymorphic microsatellite loci for pronghorn, *Antilocapra americana*. We screened 233 individuals from the National Bison Range in Moiese, MT, and found allele numbers from three to 11 and heterozygosity levels ranging from 0.142 to 0.807. These results suggest that these loci will be useful in paternity analysis and basic population genetics applications.

Keywords: *Antilocapra americana*, microsatellites, paternity analysis, pronghorn

Received 2 July 2002; revision received 10 September 2002; accepted 10 September 2002

Pronghorn, *Antilocapra americana*, are medium-sized ungulates, specialized for running in open country, endemic to western North America. They are the sole survivors of a once thriving Artiodactyl family, Antilocapridae. Historically, there has been some debate regarding the relationship between the families Antilocapridae, Cervidae and Bovidae, but current evidence suggests that pronghorn are more closely related to cervids than to bovines. Reproductively, pronghorn are unique in that females appear to have complete control over copulations, that is males cannot force matings, females mate only once per oestrus, and all females are successfully impregnated every year (Byers 1997). Male pronghorn defend either harems or territories (depending on location), and are polygynous, with a few males collecting the majority of copulations and the majority of males garnering few, if any, copulations. Although these observations are well supported by behavioural data, they have not been tested using genetic techniques.

Currently, there are five recognized subspecies of *A. americana*, two of which, sonoran pronghorn (*A. americana sonoriensis*) and peninsular pronghorn (*A. americana peninsularis*) are listed as endangered (Cancino *et al.* 1996; Woodley 1997). Microsatellite markers might be applicable in determining differences between various subspecies.

We constructed a partial genome library for *A. americana* following the protocol of Glenn (http://www.uga.edu/srel/DNA_Lab/msatmanV6.rtf). Briefly, genomic DNA (4 µg) was digested with *Sau3a*. Size selected fragments (400–1000 bp) were ligated into pBS KS (Promega), transformed into max-efficiency DH5α competent *Escherichia coli* cells

(Gibco) and plated onto Agar plates containing ampicillin, IPTG and X-Gal. A total of ≈ 3000 colonies were picked and replated to give appropriate densities (≈ 300–500 colonies on a 15-cm plate). These colonies were then lifted onto nylon membranes (GeneMate Magna Lift) and the filters were probed with CA₁₂ and GA₁₂ oligonucleotides end labelled with γ³²P-dATP. To reduce the number of false positives, positive colonies were replated and rescreened with the same probes. Double-positive colonies were cycle-sequenced in both directions using M13 forward and reverse primers. Sequence determination of resultant products was performed on an ABI 377 automated sequencer.

Approximately half of sequenced clones contained microsatellite repeats and oligonucleotide polymerase chain reaction (PCR) primers were designed for 14 loci using OLIGO Version 5.0. Eight loci were found to be polymorphic (Table 1) and six remain to be optimized. DNA used in all PCRs was extracted from pinna tissue using a QIAGEN QIAamp DNA mini kit. PCRs were performed using an Eppendorf Master Gradient Thermocycler in 20 µL total volume containing 100 ng template, 2.0 µL of 10× buffer (Invitrogen), 200 µM dNTPs, 5 pmol each primer and 0.5 units of *Taq* DNA polymerase (Invitrogen). For each locus, MgCl₂ concentration and annealing temperature were optimized separately (Table 1). The PCR profile consisted of an initial denaturation of 95 °C for 4 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at the appropriate temperature (see Table 1) for 30 s, and extension at 72 °C for 30 s, followed by a final extension period of 5 min at 72 °C.

One primer per pair for each optimized locus was resynthesized incorporating a fluorescent label, FAM, HEX or TET (Invitrogen). Resultant PCR products were screened for allelic variation on 6% denaturing polyacrylamide

Correspondence: John A. Byers. Fax: +1-208-885-7905; E-mail: jbyers@uidaho.edu

2 PRIMER NOTE

Table 1 Characteristics of eight polymorphic microsatellite loci cloned from *Antilocapra americana*. Size range (in bp) of observed allelic variation. The last two columns give observed and expected heterozygosities

Locus	Primer sequences (5'–3')	GenBank Accession no.	Annealing temp. (°C)	MgCl ₂ conc. (mM)	<i>n</i>	Repeat motif in clone	Size range	No. of alleles	<i>H_O</i>	<i>H_E</i>
<i>Aam1</i>	CCCGCGCCACAGCCAGTTCCTT GGGCCAGGGGAGGGACTCTCAA	AF525012	55	2	225	(CA) ₁₃	218–222	3	0.142	0.14
<i>Aam2</i>	CCTGCCCCCTTGCATGATTAT TAGCCACCTAACTCCCCTTCCATTG	AF525013	55	2	224	(CA) ₁₆	141–159	6	0.754	0.719
<i>Aam3</i>	TGACTCTTCTCGCTTAACTACA CAGCACCGAAGACATAGA	AF525014	50	4	218	(CA) ₁₈	163–189	7	0.661	0.736
<i>Aam4</i>	GAGCCTGGCAGGTTACAGTCTA TCCCCTCCCAATAAAAAAAGAT	AF525015	55	4	223	(CA) ₂₇	224–262	11	0.807	0.811
<i>Aam5</i>	CGGGAGGGTTCITTTACCACT GAAGGGCTTTTCAGATAGTTTCTTT	AF525016	50	2	217	(CA) ₁₁	163–169	4	0.539	0.53
<i>Aam6</i>	AGGCATTTAGTTATT TGTGTC CCCGCATCT CCTACAG	AF525017	50	2	203	(CA) ₈ CC(CA) ₂	124–130	4	0.537	0.495
<i>Aam7</i>	ATCAGCTCC CAACTCCCACTG GGCCAGCTT CCCTCAGGT	AF525018	50	2	202	(CA) ₁₅	209–217	5	0.584	0.54
<i>Aam8</i>	AGCATTTTCG CTCATTAAGAAG AGCCCTAGT GTCTAATACAGA	AF525019	45	4	217	(CA) ₁₂	194–224	7	0.742	0.743

gels using an ABI 377 with GENESCAN software. Products from all eight loci were run in a single lane by combining them post-PCR. Individual genotypes were determined using GENOTYPER (ABI) software.

All eight loci are polymorphic (Table 1) and we are currently using these markers to assess male reproductive success and patterns of genetic variation between our isolated study population and its founder population in Yellowstone National Park.

Acknowledgements

Thanks to Patryce Avsharian, Brandon Barton, Jim Latshaw, Stacy Sinor and Josh Woolsey for help in sample collection, Dr Lisette

Waits for help in analysis. Special thanks to the USFWS and the National Bison Range in Moiese, MT for access to pronghorn. This work was supported by National Science Foundation grants IBN-9808377 and DEB-0097115 to J. A. Byers.

References

- Byers JA (1997) *American Pronghorn: Social Adaptations and Ghost of Predators Past*. University of Chicago Press, Chicago.
- Cancino J, Ortega-Rubio A, Sanchez-Pacheco JA (1996) Status of an endangered subspecies: the peninsular pronghorn at Baja California. *Journal of Arid Environments.*, **32**, 463–467.
- Woodley C (1997) The sonoran pronghorn: the Air Force's strongest adversary. *Dickinson Journal of Environmental Law and Policy*, **6**, 299–319.