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Characterization of 18 microsatellite loci for prairie dogs (genus *Cynomys*)

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Abstract

We report the development of 9 new microsatellite markers and the extension of 9 existing markers for three species of prairie dogs: black-tailed (*Cynomys ludovicianus*, BTPD), white-tailed (*C. leucurus*, WTPD) and Gunnison's (*C. gunnisoni*, GUPD) prairie dogs. No consistent patterns of linkage disequilibrium were detected, and all non-founder populations were in Hardy-Weinberg equilibrium. One locus (D109) showed evidence for a null allele in BTPD and was omitted from analyses in this species. The number of alleles per locus averaged 6.0 (range 4 – 11) in BTPD, 4.5 (range 2 – 9) in WTPD, and 7.1 (range 2 – 19) in GUPD; allele sizes for six markers distinguish species. These markers will facilitate studies of disease dynamics, migration in complex landscapes, and population viability. Additionally, their utility across taxa allows for interspecific studies and will further conservation efforts.

The genus *Cynomys* comprises 5 species: the black tailed prairie dog (*C. ludovicianus*, BTPD), white-tailed prairie dog (*C. leucurus*, WTPD), Gunnison's prairie dog (*C. gunnisoni*, GUPD), Utah prairie dog (*C. parvidens*) and Mexican prairie dog (*C. mexicanus*). Through their provision of habitat for other species and their role as a food source for numerous predators, prairie dogs have pronounced effects on biodiversity. The combined distributions of the species historically covered most of the western United States, and extended into Canada and Mexico (Lomolino & Smith 2001, Miller et al. 1994). However, all five species have experienced precipitous declines due to habitat loss, shooting, poisoning, and sylvatic plague (Lomolino & Smith 2001, Hoogland 2006).

We expand the number of available microsatellite markers for characterizing genetic variation in three species of prairie dogs and describe three sets of markers that can be subjected to multiplex PCR for efficient characterization of genotypes for 18 loci (Table 1). Of the 18 markers, nine were characterized for BTPD by Jones et al. (2005). The goal of this paper is to describe markers that are widely applicable across populations and species; therefore, samples were drawn from 18 populations in three species.

Genomic libraries enriched for CA, GATC, GAAA and GGAA repeats were constructed by Genetic Identification Systems (Chatworth, CA) enrichment strategy (Jones et al. 2000). DNA fragments were ligated into pUC19 and transformed into DH5α E. coli cells. Cells were plated on selective media with X-gal and 182 inserts sequenced in both directions. Eighty sets of primers were developed for amplification. We specifically looked for primer pairs that yielded PCR product, were polymorphic in all three species, and successfully yielded interpretable genotypes when subject to multiplex PCR. The result was a set of 18 loci (approximately 10% of the microsatellite loci for which we have sequence information). Many loci we characterized but do not include in this paper are probably polymorphic in BTPD; they were excluded because they either failed to yield a PCR product in WTPD or GUPD, were not polymorphic, or did not yield interpretable genotypes using multiplex PCR. Sequences for the nine new loci were submitted to GenBank under Accession Numbers FJ971631 - FJ971639 and FJ997263 (BTPD) and FJ980459 - FJ980464 (WTPD). Information about all sequenced loci is available from the authors by request.

BTPD tissue samples were collected from 96 individuals in six colonies in Boulder County, Colorado. WTPD tissue was collected from 45 individuals from three populations in two counties in

northern and western Colorado. GUPD samples were collected throughout southwestern Colorado (145 individuals from nine colonies in four counties). DNA was extracted using a Qiagen tissue kit and amplified by multiplex PCR in 10uL reactions containing 5uL Qiagen Master Mix, 1uL primer mix, and 4uL DNA (at 5ng/uL). All reactions used MJ Research PTC-225 Peltier Thermal Cyclers. Thermocycling conditions were 15 min at 95º, followed by 34 cycles of 30 sec at 95º, 45 sec at T_a (58º for panels 1-2 and 62º for panel 3), and 30 sec at 72º, followed by 45 min at 72º, and held at 8º. Multiplex panel 1 comprised markers A119, A2, D1, D115, A105, C101, and D109; panel 2 comprised markers A101, A104, A111, D12, D2, and A109; and panel 3 comprised markers CA40-1, CA40-2, CA40-3, CA60-1, and Taga27 (Table 1). Primers were tagged with Applied Biosystems Dye Set DS-33 and run with filter set G5. Genotyping was performed on a Prism 3730 DNA Analyzer, and allele sizes were determined using GeneMapper software (both by Applied Biosystems, Inc.).

Loci were screened for null alleles using Micro-checker (van Oosterhout et al. 2004), and populations were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) in Arlequin (Schneider et al. 2000); p-values were adjusted for multiple tests of LD (0.05/15-18 loci). One locus (D109) showed evidence ($p < 0.05$) for a null allele in BTPD (this locus was included for reference but omitted from analyses of BTPD). No consistent deviations of HWE ($p < 0.05$) were found in GUPD or WTPD. Deviations from HWE in BTPD at some loci likely reflect founder effects because these colonies were recently established following plague extirpations. LD was detected in several populations, but probably reflects population structure, founder effects, admixture, or drift (Templeton 2000) because 1) patterns were not consistent across populations or species; 2) populations may be substructured due to their mating system and male-biased dispersal; 3) at least seven populations were recently founded, 4) at least six populations show evidence of potential

admixture; and 5) populations comprise small colonies that may be subject to genetic drift.

The number of alleles per locus averaged 6.0 (range 4 – 11) in BTPD, 4.5 (range 2 – 9) in WTPD, and 7.1 (range 2 – 19) in GUPD (Table 2). Allele size distribution for six loci (A104, A109, CA40-2, D1, D2, and D109) did not overlap (or only partially overlapped) among the three species; such diagnostic loci may be useful in identifying samples of unknown origin. Characterization of these markers will aid in future studies of prairie dogs, such as assessing migration in varied landscapes, inbreeding in isolated populations, paternity, and the genetic effects of disease.

the fact that the other 12 did would be further evidence that we don't expect subspecies to differ greatly

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Table 1. Microsatellite locus primer sequences and multiplex panel groupings for prairie dogs (genus *Cynomys*). Asterisks represent loci originally developed by Jones et al. 2005.

Locus	Forward primer (5' - 3')	Reverse primer (5' - 3')	panel
*A119	GTGGCAAGAAAGGGGTAAA	GCTCTGTGTGTGTGTGTGTATG	1
*A2	CCATTCTACATCCCAGGAG	AGCCAGTATGATTTAGGTGGT	1
*D1	ACCTTTTGTTTCATTCTCAGC	TGCCATAGTTTGCTTTCTTACT	1
*D115	CAGGCATCTATGGAAGACAG	CTTTGATTGGTGAGTTTTGTG	1
A105	GGTGAGGTGGTGTAGGAAGG	TGTTGGCATCTAGTTGAGTGG	1
C101	GCTGCCGTTTCTGAAAACTT	CTCCTCCTCAACCACCTGAG	1
D109	TCTGAGCTTGGGGGAACTTA	TCAGACTTGCTGAACCTCCA	1
*A101	ACTGCCAAATGCATAATTTC	CCAACTTAGGTGCCCTACAAC	2
*A104	TCAGGGACAGAAAAAGAACTT	TATTTGGGCATCATTCAGTC	2
*A111	TCCCACTCTACTTAGCAAAAAT	CCTACCTCGTCTTAAAAAATTG	2
*D12	TTACCTCCCCACACACAAA	TGCCTCACTATTGGACAGC	2
*D2	TTGAAGAAGTAGCACAGTGGTA	GGAGATAAAGGAGACAAGAACA	2
A109	CCCCAAAGATTCTGGACTTG	TGGATGGAATCTTCCAGGAG	2
CA40-1	CAACATGCATGCCCAAGAT	TCACCAGACTGTTACTTTGGCTA	3
CA40-2	CATCTGCCTGGTGTATTCTCTG	TTTTAGAGGTTGCCCACCAG	3
CA40-3	CTCTTGCTCCCAGGACTCAG	GGGAGGAGAGTGAGGGAAAG	3
CA60-1	TAACTGGGATGCCTCCACTC	TGTGAGTCCAACATGTTAATTACTGA	3
Taga27	ATAGGGCTGGGGATACTGCT	GCTCTCACCAGAGCCTGAAC	3

* from Jones et al. 2005

Table 2. Number of alleles and size range of each locus in three species of prairie dogs (BTPD: black-tailed prairie dog; WTPD: white-tailed prairie dog; GUPD: Gunnison's prairie dog). N is the average number of individuals genotyped per locus; N/A indicates no amplification at the locus.

		Locus								
		A101	A104	A105	A109	A111	A119	A2	C101	CA40-1
Species	N	number of alleles size range								
BTPD	96	7 127-139	6 172-186	5 188-196	6 305-323	5 172-180	6 98-120	5 207-217	11 279-323	6 247-259
WTPD	45	3 121-125	5 162-168	2 193-197	8 309-311; 341-365	3 176-180	4 104-112	3 199; 219-221	7 315; 331-351	5 251-261
GUPD	145	7 121-141	6 162-174	4 193-199	13 327-357	5 174-184	6 108-120	9 199-227	17 297-341	N/A
total # alleles		**11**	**13**	**9**	**22**	**6**	**10**	**11**	**28**	**8**

		Locus								
		CA40-2	CA40-3	CA60-1	D1	D109	D115	D12	D2	Taga27
Species	N	number of alleles size range								
BTPD	96	6 170-198	6 193-207	7 235-247	4 182-194	9 410-434	4 194-210	5 190-206	8 282-310	5 206-222
WTPD	45	4 162-180	4 197-211	N/A	2 168-184	4 407-419	4 194-206	4 198-210	5 304-324	9 206-242
GUPD	145	6 172-184	6 195-207	N/A	1 184	N/A	7 186-210	6 186-206	6 294-314	8 204-238
total # alleles		**11**	**9**	**7**	**6**	**13**	**7**	**7**	**14**	**11**