

PARENTAGE, MULTIPLE PATERNITY, AND BREEDING SUCCESS IN GUNNISON'S AND UTAH PRAIRIE DOGS

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Feasibility of assigning parentage using variable microsatellite loci was assessed for 2 species of prairie dogs. Parentage was determined from 7 microsatellite loci for 46% of juveniles born during 1994 in a colony of Gunnison's prairie dogs (*Cynomys gunnisoni*), and for 53% and for 45% of juveniles born during 1996 and 1997, respectively, in a colony of Utah prairie dogs (*C. parvidens*). Frequency of multiple paternity estimated for Gunnison's (77%) and Utah (71% and 90%) prairie dogs was greater than that detected previously for black-tailed prairie dogs (5%–10%) but within the range reported for other ground-dwelling squirrels. Of the 84 adult females and 33 adult males present during 1994 in the colony of Gunnison's prairie dogs, 75 (89%) and 22 (67%), respectively, produced weaned offspring. Breeding success for Utah prairie dogs was relatively low in 1996 (45% for females and 32% for males) but increased in 1997 (80% for females and 81% for males).

Key words: Breeding success, *Cynomys*, Gunnison's prairie dog, microsatellites, multiple paternity, Utah prairie dog

Behavioral ecologists have documented the importance of social systems on genetic structure, inbreeding, and reproductive success (Chesser 1998; Dobson 1998; Dobson et al. 1998; Long et al. 1998; Pope 1998; Sugg et al. 1996). Unfortunately, estimating these and other demographic characteristics from behavioral observations is difficult and often misleading. Paternity can be particularly difficult to determine, for example, when organisms have large home ranges (Schenk and Kovacs 1995), underground or underwater copulation (Coltman et al. 1998; Hoogland 1995; Taylor et al. 1997), or multiple mates per estrus female (e.g., Hanken and Sherman 1981; Hoogland 1995, 1998a; Robinson 1982).

Prairie dogs (*Cynomys*) are colonial, diurnal, burrowing rodents of the squirrel family (Sciuridae), and inhabit open grasslands throughout the western United States and northern Mexico (Hoogland 1995). They have been the subject of numerous studies focusing on social organization and genetic structure of colonial mammals (Dobson et al. 1998; Hoogland 1995; Sugg et al. 1996; Travis et al. 1996). Of the 5 species of prairie dogs, black-tailed prairie dogs (*C. ludovicianus*) have been studied most intensively (Chesser 1983a, 1983b; Dobson et al. 1998; Hoogland 1995; King 1955). Recently, however, long-term behavioral and genetic studies have been initiated to better understand social structure of Gunnison's prairie dogs (*C. gunnisoni*; Hoogland 1996, 1997, 1998a, 1998b, 1999; Trav-

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is et al. 1995, 1996, 1997) and Utah prairie dogs (*C. parvidens*; Hoogland 2001).

Gunnison's prairie dogs occur in south-eastern Utah, northwestern New Mexico, northeastern Arizona, and southwestern Colorado. Utah prairie dogs, found in south-central Utah, currently are on the U.S. list of species threatened with extinction (Hoffmann et al. 1993), and are under consideration for the list of endangered species. Female Gunnison's prairie dogs become sexually mature during their 1st year, whereas males often do not become sexually mature until their 2nd year (Hoogland 1997; Rayor 1985, 1988). Females annually enter a 1-day estrus, mate with 1–5 males (Hoogland 1998a, 1998b), and eventually wean 1–7 offspring (Hoogland 1998a). Utah prairie dog females also come into estrus once a year, both males and females usually mate with multiple partners, and females produce litters of 1–7 young (Hoogland 2001). Females of both species usually mate, and always give birth, underground. Observation of copulation and parturition is therefore difficult (but see Hoogland 1998a, 1998b, 2001).

Social organization of Gunnison's and Utah prairie dog colonies is similar to that of black-tailed prairie dogs. Specifically, a colony is subdivided into social units called clans that usually contain several adult females, their offspring, and 1–2 breeding males (Hoogland 1999). Membership within clans of Gunnison's and Utah prairie dogs, however, is less rigid than membership within social groups (called coterie) of black-tailed prairie dog colonies. Although adult Gunnison's and Utah prairie dog males usually associate with 1 clan, these males commonly breed with females of neighboring clans.

The primary purpose of this study was to test the feasibility of using a combination of behavioral observations and variable microsatellite loci to document parentage for all juveniles born within a Gunnison's prairie dog colony in 1994 and a Utah prairie dog colony in 1996 and 1997. Once par-

entage was determined, for each litter we examined frequency of multiple paternity and breeding success of males and females. If we determined that sufficient numbers of parentage assignments could be made with these data, the 2nd objective was to test the hypothesis that the looser social structure exhibited by Gunnison's and Utah prairie dogs, relative to the coterie system of black-tailed prairie dogs, would lead to increased levels of multiple paternity.

MATERIALS AND METHODS

Blood was collected from all potentially breeding individuals (i.e., those individuals observed displaying breeding behaviors and considered as potential parents) and young in a Gunnison's prairie dog colony (Petrified Forest National Park, Apache County, Arizona) sampled during 1994 ($n = 380$) and a Utah prairie dog colony (Bryce Canyon National Park, Garfield County, Utah) sampled during 1996 ($n = 147$) and 1997 ($n = 225$). Methods of capture, blood sampling, and collection of behavioral data are similar to those described by Hoogland (1995, 1997).

Genomic DNA was extracted from 50 μ l of whole blood following the method of Longmire et al. (1997). Microsatellite loci were amplified via the polymerase chain reaction (PCR) with previously published primers developed by Stevens et al. (1997) from Columbian ground squirrels (*Spermophilus columbianus*). Primers for all loci were redesigned to allow multiplex gel loading (Table 1).

PCR amplifications were conducted in 15- μ l volumes containing 50 ng genomic DNA, 10 pmols each primer, 9 μ l True Allele Premix (Perkin-Elmer Applied Biosystems, Foster City, California), and 3.8 μ l double distilled H_2O . The thermal profile consisted of a denaturation and enzyme activation cycle at 95°C (12 min); 10 cycles of 94°C (15 s) denaturation, 55°C (60 s, 52°C for Utah prairie dogs) annealing, and a 72°C (30 s) elongation; followed by 25 cycles of 89°C (15 s) denaturation, 55°C (60 s) annealing, 72°C (30 s) elongation. A final incubation at 72°C (30 min) was used to ensure that all reactions had gone to completion. Variation at individual microsatellite loci was visualized using an automated DNA sequencer (model 377, Perkin-Elmer Applied Biosystems, Foster City,

TABLE 1.—Locus name and PCR primers used for analysis of Gunnison's prairie dog (*Cynomys gunnisoni*) and Utah prairie dog (*C. parvidens*) populations. The name of each locus is as originally described by Stevens et al. (1997); however, all primer sequences were redesigned to allow multiplex gel loading.

Locus	Forward primer	Reverse primer
GS08	HEX-ACCAATGGGAGACACATCCAA	GTGTCTTAACTCCTTGTAATAGCCCCCTG
GS12	NED-CCAAGAGAGGCAGTCGTCCAG	GTGTCTTTCGAGCAGAGCACTTACAGA
GS14	6FAM-CAGAATCAGGTGGGTCCATAGTG	GTGTCTTGATGAAACCTATTTGCCTTCCTTC
GS17	6FAM-CAATTCGTGGTGGTTATATC	GTGTCTTCTGTCACCTATATGAACACA
GS20	6FAM-GCCAGCCATCACCCTCACC	GTGTCTTTCAGAGTTTTTCAGACACA
GS22	6FAM-AGAGAACAACATCATCAACAGGGTGTG	GTGTCTTGGTCTCATCTGCCAATTTTC
GS26	NED-GGCTCCAAGTCCCAGGGAC	GTGTCTTGGTCTCATCTGCCAATTTTC
GS34	NED-CTTTCTTCTGCTCTGTTATC	GTGTCTTACCTCACTTTATCTCTGAA

California). Amplicons for each locus from a single individual were mixed (0.5 μ l each PCR product) and 1 μ l of this mixture was combined with 3 μ l of loading mix (2.5 μ l formamide, 0.5 μ l ROX size standard, 0.25 μ l loading buffer containing blue dextran). The PCR-loading dye mixture was denatured at 95°C (5 min) and 1.5 μ l was loaded into a single lane of a 5% polyacrylamide gel. All samples from juveniles were run on the same gel with potential mothers and fathers. Genotypes were visualized using GENESCAN and GENOTYPER software (Perkin-Elmer Applied Biosystems, Foster City, California).

Data Analysis

Marker analysis.—CERVUS 1.0 software (Marshall et al. 1998) was used for computation of allele frequencies, expected and observed heterozygosity, frequency of null alleles, polymorphic information content (PIC-index of variability), and 2 exclusion probabilities for parentage assignment. Probability of identity (PI-probability of randomly selecting 2 individuals with identical genotypes from a population) for each locus and for all variable loci was calculated as described by Paetkau and Strobeck (1994).

When performing parentage analyses based on genetic exclusion, true parent-offspring relationships were rejected if the genotype of the adult could not produce the observed genotype of the offspring. Such "mismatches" between the true parent and offspring can be due to "null" alleles, mutations, and polymerase stutter (a technical artifact). Non-amplifying, or "null," alleles at microsatellite loci occur frequently in humans (Callen et al. 1993), deer (*Cervus elaphus*; Pemberton et al. 1995), and bears (*Ursus*

americanus, *U. thibetanus*; Paetkau and Strobeck 1995) and therefore consideration must be given to the existence of such alleles in any study using microsatellites. Presence of segregating null alleles in populations is thought to be the result of sequence polymorphisms that affect the binding site in 1 of the oligonucleotide primers used in amplification (Paetkau and Strobeck 1995). Mismatches between offspring and at least 1 of the parents due to null alleles can be detected by offspring appearing homozygous for an allele detected in only 1 parent. In contrast, mismatches due to mutations or stutter of the polymerase during amplification of the microsatellite locus result in the offspring possessing an allele not detected in either parent.

Parentage.—Pregnant and lactating female prairie dogs typically guard their nursery burrows from all other females (Hoogland 1995, 1997). Thus, maternity usually can be assigned to the female guarding and using the burrow from which juveniles 1st emerge, about 11 weeks after copulation. The number of burrows was limited in the Gunnison's prairie dog colony in 1994, however, forcing some females to share burrows. Determination of maternity based on observation was therefore difficult (see also Rayor 1988). In these cases, all females observed using the same burrow were considered potential mothers of juveniles 1st emerging from that burrow. Although observational assignments of maternity were easy for Utah prairie dogs in both 1996 and 1997, all assignments were checked using the approach described below. For both species, potential fathers were assigned to each juvenile by observing which males displayed behaviors indicative of copula-

tion (Hoogland 1998a, 1998b) with the female(s) guarding a particular nursery burrow.

All females and males determined to be potential parents based on observational data were submitted to the following analyses in order to reduce the number of individuals used in the final parentage analysis. Candidate parents were first compared to potential offspring using genetic exclusion methods. Genetic exclusion of individuals as biological parents was evident when neither allele in a juvenile's diploid genotype matched an allele of the candidate in question for 1 or more loci. In addition to genetic exclusion, genetic likelihood was calculated (using as a baseline the allele frequencies in the population) from joint genotypic frequencies observed in particular combinations of potential parents and juveniles. Scores for likelihood ratio between parent-offspring status and unrelatedness (LOD scores) were calculated for all potential parent-juvenile dyads. Confidence for initial assignments was based on Δ LOD (Δ LOD = LOD of most-likely parent minus LOD of next most-likely parent). Likelihood calculations were carried out using CERVUS software (parameters shown in Table 2).

Only females and males considered to be potential parents based on observational data, not excluded as potential parents during exclusion analyses, and determined to be potential parents during likelihood analyses were used in the final parentage analysis. For each juvenile, all possible combinations of candidate mothers and candidate fathers were considered, with adult females being considered the known parents and adult males the candidate parents. Using exclusion methods, mother-juvenile dyads were compared to all candidate fathers. Paternity was assigned only to adult males that possessed multilocus genotypes compatible with producing the multilocus genotype observed in juveniles based on mother-juvenile dyads. Genetic likelihood also was calculated using joint genotypic frequencies observed in particular combinations when mother-juvenile dyads were compared to all candidate fathers. Both number of mismatches between mother-juvenile-father triads and LOD scores were considered when assigning parentage. Confidence for parentage assignments was based on Δ LOD scores. Final parentage was assigned to the male and female pair with the highest LOD scores and the fewest mismatches with the juvenile.

TABLE 2.—Parameters used during likelihood analyses with CERVUS software (Marshall et al. 1998) for populations of Gunnison's prairie dog (CERVUS, Marshall et al. 1998) and Utah prairie dog (1996 and 1997). Parameters are given for initial maternity and paternity analyses (prior to parentage analysis) and for the final parentage analysis. Because males were considered the candidate parents, parameters for paternity analysis and parentage analysis were the same. Parameter values include data from locus GS22.

	Number of simulation cycles	Number of candidate parents	Proportion of candidates sampled	Proportion of loci typed	Proportion of loci mistyped	Confidence levels
Gunnison's, 1994 (maternity)	100,000	84	0.880	0.923	0.030	95, 80, 65, 50
Gunnison's, 1994 (paternity, parentage)	100,000	33	0.909	0.923	0.030	95, 80, 65, 50
Utah, 1996 (maternity)	100,000	42	1.000	0.947	0.030	95, 80, 65, 50
Utah, 1996 (paternity, parentage)	100,000	31	1.000	0.947	0.030	95, 80, 65, 50
Utah, 1997 (maternity)	100,000	46	1.000	0.943	0.030	95, 80, 65, 50
Utah, 1997 (paternity, parentage)	100,000	31	1.000	0.943	0.030	95, 80, 65, 50

Multiple paternity.—Once parentage was determined, juveniles were assigned to litters based on maternity. Those juveniles with undecided maternity were not assigned to litters and were not included in the multiple paternity analysis. Multiple paternity calculations considered only those litters that had ≥ 2 juveniles. A litter was considered to have multiple sires when ≥ 2 juveniles had different fathers or different potential fathers remaining after paternity assignment. Frequency of multiple paternity was calculated by dividing the number of litters sired by ≥ 2 males by the total number of litters that contained ≥ 2 juveniles.

RESULTS

Gunnison's Prairie Dogs—1994

Based on observational data, the study colony consisted of 20 clans containing 84 potentially breeding adult females, 33 potentially breeding adult males, and 263 juveniles. All 380 individuals were genotyped for at least 1 locus with the exception of 2 adult females. Number of alleles per locus ranged from 2 to 6 with a mean of 4.29 (Table 3). Based on PIC and PI, loci GS08, GS14, and GS22 were most informative and GS17 and GS20 were least informative (Table 3). First-parent exclusionary power was 77%. However, 2nd-parent exclusionary power (i.e., the ability to exclude males as potential fathers when the mother was known) was 95%.

Parentage.—Two juveniles were removed from analyses due to lack of observational data for comparisons. An additional problem encountered was polymerase stutter associated with dinucleotide repeats. Although locus GS22 was one of the most informative loci, this locus frequently contained many additional bands due to stutter during amplification. This polymerase stutter prevented consistent scoring of individuals for this locus and may have contributed to its tentative rank as a highly informative locus. Therefore, due to difficulties with consistently scoring GS22, parentage was reassessed after removing this locus and any individuals that were previously re-

TABLE 3.—Locus name and descriptive statistics for genetic variation at each locus for a population of Gunnison's prairie dogs (*Cynomys gunnisoni*) collected from the Petrified Forest National Park, Apache County, Arizona, 1994, and a population of Utah prairie dogs (*Cynomys parvidens*) collected from Bryce Canyon National Park, Garfield County, Utah, 1996–1997. A = number of alleles, n = sample size, H_o = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphic information content, PE1 and PE2 are first- and second-parent exclusionary probabilities, respectively, and P1 = probability of identity (Paetkau and Strobeck 1994). An (—) indicates that there are no data for that locus in that population.

Locus	Gunnison's prairie dogs, 1994							Utah prairie dogs, 1996							Utah prairie dogs, 1997									
	A	n	H _o	H _E	PIC	PE1	PE2	PI	A	n	H _o	H _E	PIC	PE1	PE2	PI	A	n	H _o	H _E	PIC	PE1	PE2	PI
GS08:	5	321	0.636	0.696	0.647	0.276	0.450	0.140	4	145	0.369	0.647	0.574	0.211	0.359	0.197	4	222	0.342	0.630	0.557	0.199	0.343	0.210
GS12:	6	331	0.363	0.492	0.473	0.139	0.310	0.273	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
GS14:	6	352	0.642	0.804	0.773	0.430	0.608	0.072	3	145	0.014	0.014	0.014	0.000	0.007	0.973	3	225	0.009	0.009	0.009	0.000	0.004	0.983
GS17:	2	370	0.165	0.169	0.155	0.014	0.077	0.705	2	145	0.007	0.007	0.007	0.000	0.003	0.987	2	225	0.004	0.004	0.004	0.000	0.002	0.991
GS20:	2	327	0.076	0.134	0.125	0.009	0.062	0.759	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
GS22:	5	377	0.305	0.686	0.631	0.267	0.433	0.154	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
GS26:	4	378	0.437	0.453	0.411	0.106	0.245	0.341	2	145	0.386	0.401	0.320	0.080	0.160	0.440	2	224	0.317	0.364	0.297	0.006	0.149	0.471
GS34:	—	—	—	—	—	—	—	—	4	111	0.441	0.618	0.562	0.205	0.367	0.200	4	145	0.441	0.627	0.576	0.214	0.381	0.190
Mean:	4.29	—	0.375	0.490	0.459	0.772	0.945	7.7 × 10 ⁻⁵	3.0	—	0.243	0.339	0.295	0.423	0.663	7.7 × 10 ⁻⁵	3.0	—	0.223	0.327	0.155	0.412	0.656	1.8 × 10 ⁻²

TABLE 4.—Summary of parentage assignments for populations of Gunnison's prairie dogs in 1994, and Utah prairie dogs in 1996 and 1997. Complete parentage indicates that both maternity and paternity were resolved, paternity only indicates that only paternity was resolved, maternity only indicates that only maternity was resolved, and unresolved indicates that neither maternity nor paternity was resolved.

	Complete parentage	Paternity only	Maternity only	Unresolved
Gunnison's, 1994	120	18	98	25
Utah, 1996	40		35	
Utah, 1997	67		81	

moved due to a mismatch at GS22 were included as potential parents.

Because females were considered the known parents, maternity assignments were made before performing paternity analyses. Based on a combination of behavioral, exclusion, and likelihood methods, we were able to assign maternity to 218 of 261 (84%) juveniles in 1994. Maternity was ambiguous (>1 possible female) for 43 of 261 (16%) juveniles.

Final maternity and paternity assignments were made using a combination of behavioral data, exclusion, and likelihood methodologies. Complete parentage was assigned to 120 of 261 (46%) juveniles involved in the analyses (Table 4). For the 141 juveniles for which complete parentage was not assigned, some individuals were removed as potential parents but exact parentage could not be determined. For 18 of these juveniles, only paternity could be determined because each had >1 female remaining as potential mothers. For an additional 98 juveniles only maternity could be determined. Among these 98 juveniles for which only maternity could be determined, paternity could not be determined for 49 because >1 male remained as a potential father, whereas all potential fathers were excluded due to mismatches with either the juvenile or the mother-juvenile dyad for the remaining 49 juveniles. For the remaining 25 juveniles, neither maternity nor paternity could be determined.

Multiple paternity.—Seventy-five litters comprising 218 juveniles were identified from parentage analyses. Sixty-three litters

had ≥ 2 juveniles. Complete parentage was determined for all juveniles within a litter or paternity was determined for ≥ 2 juveniles assigned to that litter for 44 of these 63 (70%) litters. Ten of 44 (23%) litters were sired by a single male, while 34 (77%) showed unequivocal multiple paternity.

Breeding success.—We define breeding success as the percentage of adult female and male prairie dogs that successfully copulate and produce ≥ 1 juvenile. Seventy-five of 84 (89%) adult females thought to be potential breeders produced at least 1 offspring. These 75 females produced litters ranging in size from 1–6 juveniles (mean \pm SE = 2.91 ± 0.14). Paternity was unambiguously resolved for 138 of 261 (53%) juveniles. Therefore, of the 33 breeding males sampled during 1994, 22 (67%) contributed genes to the next generation, with the number of juveniles sired per successful male ranging from 1–17 (mean \pm SE = 6.27 ± 0.79).

Utah Prairie Dogs—1996 and 1997

The Utah prairie dog colony sampled during 1996 was divided into 13 clans containing 42 adult females, 31 adult males, and 75 juveniles. With the exception of 3 juveniles that were genotyped at ≤ 2 loci, all individuals were genotyped ≥ 5 loci. Although maternity was assigned based on behavioral observations, these 3 juveniles were removed from subsequent paternity analyses. During 1997, this same prairie dog colony was divided into 14 clans containing 46 adult females, 31 adult males, and 148 juveniles. All individuals except 2

were genotyped for ≥ 5 loci. One adult male could be genotyped only at 4 loci and 1 juvenile could be genotyped only at 3 loci. No individuals were removed from subsequent parentage analyses.

Number of alleles per locus ranged from 2 to 4 with a mean of 3.0 for both 1996 and 1997 (Table 3). Based on PIC and PI, loci GS08 and GS34 were most informative for both years (Table 3). Locus GS20 was fixed for 1 allele, and the same was essentially true for GS14 and GS17. Locus GS12 was anomalous because all individuals were scored as heterozygotes. Loci GS12 and GS20 were removed from subsequent analyses. Exclusion probabilities decreased slightly from 1996 to 1997 (Table 3).

Parentage.—Maternity was assigned to all 75 juveniles born in 1996 based on behavioral data, as there was only 1 potential mother assigned to each juvenile. However, 1 female–juvenile dyad possessed a mismatch at locus GS34 that may be explained as a mutation. In 4 other instances, mismatches caused by null alleles occurred between juveniles and their respective mothers. For the remaining 70 juveniles, no mismatches occurred.

Maternity was assessed for all 148 juveniles born in 1997. As with the 1996 data, maternity was assigned to all juveniles based on behavioral data. Two female–juvenile dyads possessed mismatches at locus GS34. In both instances, the dyads were supported with a “most-likely” confidence value by CERVUS. In 6 other instances, mismatches due to the apparent presence of null alleles occurred. In the remaining 140 instances, no mismatches occurred.

Complete parentage was assigned to 40 of 75 (53%) juveniles born in 1996 (Table 4). The remaining 35 juveniles had maternity assigned, but either all potential fathers were excluded ($n = 4$) or paternity was ambiguous ($n = 31$), with ≥ 2 males as potential fathers. Parentage was assigned to 67 of 148 (45%) juveniles born in 1997 (Table 4). For the remaining 81 juveniles, maternity was assigned but either all potential fathers

were excluded ($n = 2$) or paternity was ambiguous ($n = 79$), with ≥ 2 males remaining as potential fathers.

Multiple paternity.—For the Utah prairie dogs sampled during 1996, there were 19 litters encompassing all 75 juveniles and all but 1 litter comprised ≥ 2 juveniles. Fourteen of 18 litters (78%) had complete parentage assigned to ≥ 2 juveniles. Ten of 14 litters (71%) were determined to be multiply-sired, while 4 were sired by a single male. For this same colony sampled in 1997, 37 litters encompassing all 148 juveniles were determined. Thirty-five litters had ≥ 2 juveniles, of which 19 (54%) had complete parentage determined for ≥ 2 juveniles. Seventeen of 19 litters (90%) were sired by ≥ 2 males.

Breeding success.—For the colony of Utah prairie dogs sampled in 1996, only 19 of the 42 (45%) females of breeding age displayed copulatory behavior and produced litters. Litter size ranged from 1–5 juveniles (mean $\pm SE = 3.95 \pm 0.26$). For this same colony sampled in 1997, 37 of 46 (80%) females of breeding age produced litters, ranging in size from 1–7 juveniles (mean $\pm SE = 4.00 \pm 0.22$). For the colony of Utah prairie dogs sampled in 1996, only 10 of 31 (32%) potentially breeding males sired young. Paternity was resolved for 40 of 75 (53%) juveniles, with the number of juveniles sired per male ranging from 1–7 (mean $\pm SE = 4.44 \pm 0.78$). All 25 males were still considered potential fathers of ≥ 1 juvenile. Paternity was resolved for 67 of 148 (45%) juveniles, with the number of juveniles sired per male ranging from 1–8 (mean $\pm SE = 3.53 \pm 0.37$).

DISCUSSION

In most studies of behavioral ecology, maternity is determined from observational data, but paternity usually must be determined from molecular methods. Kanthaswamy and Smith (1998) were able to assign paternity to 127 of 129 (98%) rhesus macaques (*Macaca mulatta*). Coltman et al. (1998) however, were able to assign pater-

nity to only 85 of 275 (31%) juvenile harbor seals (*Phoca vitulina*) over a 2-year period. Similarly, Petri et al. (1997) and Keane et al. (1997) reported success rates of 37% and 55% for the large mouse-eared bat (*Myotis myotis*) and toque macaques (*Macaca sinica*), respectively. As with many parentage studies, we could not assign parentage to all juveniles in either study population. We unambiguously assigned parentage to 120 of 261 (46%), 40 of 75 (53%), and 67 of 148 (45%) juveniles in the colony of Gunnison's prairie dogs sampled in 1994 and the colony of Utah prairie dogs sampled in 1996 and 1997, respectively.

Multiple paternity and breeding success.—Multiple paternity has been documented in several other ground-dwelling squirrels including California ground squirrels (*Spermophilus beecheyi*; Boellstorff et al. 1994), Belding's ground squirrels (*Spermophilus beldingi*; Hanken and Sherman 1981), Columbian ground squirrels (Murie 1995), and black-tailed prairie dogs (Hoogland 1995). Frequency of multiple paternity for Gunnison's and Utah prairie dogs was markedly higher than the 5–10% reported for black-tailed prairie dogs (Hoogland 1995), but lower than or similar to the 89% reported for California ground squirrels (Boellstorff et al. 1994).

Although multiple mating and multiple paternity appear to be common in ground-dwelling squirrels (Hanken and Sherman 1981; Hoogland 1995; Murie 1995), the adaptive significance of both are not well understood. One possibility is that multiple mating in Gunnison's and Utah prairie dogs ensures insemination. Probability of conception and parturition in Gunnison's prairie dogs, for example, was 100% when females mated with ≥ 3 males, but only 92% when females mated with ≤ 2 males (Hoogland 1998a). In addition, litter size varies directly with the mother's number of different sexual partners (Hoogland 1998a).

Despite availability of detailed behavioral observations and all relevant blood sam-

ples, parentage could not be assigned to all juveniles in any single year. Two factors hindered our assignments. First, although the loci we examined are highly variable in other ground-dwelling squirrels (Stevens et al. 1997), they exhibit less variability for Gunnison's and Utah prairie dogs. The most likely reason for the low variability is that Gunnison's and Utah prairie dogs are rare, and both have been (Gunnison's prairie dogs) or are (Utah prairie dogs) in serious danger of extinction. Second, for several juveniles, all potential males were removed during paternity analyses. In the Gunnison's prairie dog colony, for example, exclusion of all possible males occurred for 49 of 261 (18.8%) juveniles during paternity assignments.

The primary purpose of this study was to evaluate resolving power of our chosen genetic loci for Gunnison's and Utah prairie dogs. Despite the difficulties outlined above, we were able to deduce enough parentages to allow estimates of the frequency of multiple paternity and breeding success. To address questions and problems raised by this study, we currently are genotyping the same Gunnison's prairie dog colony sampled from 1991–1993 and the same Utah prairie dog colony sampled from 1998–2001. We also are searching for additional microsatellite loci. We anticipate that additional microsatellite loci will aid in the determination of more parental assignments. Furthermore, we are developing multigenerational pedigrees for both species of prairie dogs. Such multigenerational pedigrees will allow us to assess social and genetic structure, multiple paternity, breeding success, reproductive success, and inbreeding (Dobson et al. 1998). Further, extended pedigrees will allow more accurate determination of the relatedness of males associated with a clan, the relatedness of females in adjacent clans, and the extent to which specific males monopolize mating opportunities within clans.

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