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ANALYSIS OF THE MATING SYSTEM IN THE BLACK-TAILED PRAIRIE DOG (*CYNOMYS LUDOVICIANUS*) BY LIKELIHOOD OF PATERNITY

DAVID W. FOLTZ AND JOHN L. HOOGLAND

ABSTRACT.—Black-tailed prairie dogs (*Cynomys ludovicianus*) live in colonies composed of contiguous but separate groups called coteries. A coterie usually contains one or two adult males, one to six adult females, and several yearlings and juveniles. For 50 of 52 litters produced by 46 females in 1979 and 1980, an electrophoretic analysis of four blood proteins indicated that the litter was fathered by one of the males in the home coterie. For 14 of 18 litters produced in coteries containing more than one adult male, paternity could be unambiguously assigned to one of the resident males. These results indicate that coteries, originally defined as units of social structure, are also units of reproduction.

Populations of several species of sciurid rodents are organized into groups composed of one or two adult males and one or more adult females. These include four species of *Marmota* (Barash, 1973, 1974, 1976; Downhower and Armitage, 1971) and *Spermophilus columbianus* (Steiner, 1970). Perhaps the most elaborate social organization among rodents is found in the black-tailed prairie dog (*Cynomys ludovicianus*), a diurnal, colonial species inhabiting dry upland prairies (King, 1955). Within a colony, individuals live in groups known as coteries. A coterie typically contains one or two adult males, one to six adult females, and several yearlings and juveniles of both sexes. Males and females usually first breed at 2 years of age. Individuals usually remain within well-defined continguous coterie territories, are amicable toward members of their own coterie, and are hostile toward members of other coteries (King, 1955; Hoogland, 1981*b*).

The assumption often is made (e.g., Downhower and Armitage, 1971) that social groups of rodents also constitute reproductive units, although copulations are observed rarely or not at all. The lack of information is particularly acute for black-tailed prairie dogs, because copulation usually occurs in the burrow (Hoogland, 1981*b*). The technique of gel electrophoresis has made it possible in some instances to supplement behavioral observations by obtaining genetic information for all members of a social group. For example, with data from eight polymorphic loci, Schwartz and Armitage (1980) reported that females within harems of yellow-bellied marmots (*Marmota flaviventris*) bred with resident harem males (see also McCracken and Bradbury, 1977; Hanken and Sherman, 1981). The purpose of our study was to determine from an electrophoretic analysis of blood protein variation if female prairie dogs within a coterie usually breed with resident coterie males.

MATERIALS AND METHODS

The study colony.—The study colony was located in Wind Cave National Park, Hot Springs, South Dakota. This colony occupied an area of 6.6 ha, and contained approximately 130 adults (animals 2 years old or older) and yearlings each year arranged in approximately 25 coteries (Hoogland, 1981*a*). The nearest other colony was 0.7 km away. In South Dakota, mating occurred from late February through early April, and weaned juveniles first emerged from their natal burrows in May and June; mean (±SD) litter size was 2.90 ± 1.07 (Hoogland, 1981*b*). Coterie compositions were determined by behavioral observations (King, 1955; Hoogland, 1981*b*).

Marking techniques.—Since 1975, all residents in the study colony were marked with numbered ear-tags for permanent identification and with fur dye for visual identification (Hoogland, 1979, 1981*b*). The mother of each litter could be identified reliably because, during lactation, the mother slept with her litter at night and defended the burrow containing her young during

the day. Young were marked before they mixed with young from other litters, so that exact mother-offspring and sibling-sibling relationships could be determined.

Electrophoretic analysis.—In 1979 and 1980, blood samples were collected from all prairie dogs in the study colony. Blood obtained by cutting one or more foot pads with a lancet was collected in heparinized capillary tubes and centrifuged at room temperature for 10 min in a micro-hematocrit centrifuge. The plasma and erythrocyte fractions were transferred to separate plastic vials and stored at −70°C or lower. The method of horizontal starch-gel electrophoresis followed the procedure of Selander et al. (1971), except for the nucleoside-phosphorylase stain, which was modified from that of Edwards et al. (1971). In blood samples collected in 1979 and stored for 1 year before electrophoresis, 6-phosphogluconate dehydrogenase had lost most of its activity and could not be scored. However, other polymorphic proteins showed no evidence of deterioration during storage, when compared with proteins in blood samples collected from the same animals in 1980 and stored for only 2 months before electrophoresis.

Statistical analysis.—Our method was based on the work of Thompson (1976), who made the first detailed application of likelihood methods to genealogical inference. Assumptions on which the method is based are that (1) each polymorphism is controlled by codominant alleles at a single autosomal locus, (2) the loci obey the Mendelian laws of segregation and independent assortment, (3) all copulations resulting in offspring are performed by adult males resident in the study colony, (4) the genotype of each adult male is known for all loci examined, and (5) each litter is the result of insemination by only one male. In contrast to other methods for analyzing paternity (Birdsall and Nash, 1973; Foltz, 1981), it is not necessary to assume random mating.

Consider a litter containing N offspring born to female F. The likelihood that male M is the father of F's entire litter is the probability of obtaining the observed offspring genotypes if male M is the father. For one locus, assume that the offspring were sorted into c categories according to genotype. If n_i denotes the number of offspring in the i^{th} genotypic category, then for this locus the likelihood of paternity for male M is

$$\frac{N!}{\prod_{i=1}^{c} n_i!} \prod_{i=1}^{c} (P_i)^{n_i},$$

where P_i is the probability of obtaining the i^{th} genotype from the mating of F and M. The combined likelihood of paternity for several independently assorting (unlinked) loci is found by multiplying the individual likelihoods. It is conventional to transform the combined likelihoods by taking the natural logarithm (Edwards, 1972); these log-likelihoods will be symbolized by L. Log-likelihoods may range from 0 to $-\infty$; higher values of L (those closer to 0) indicate higher likelihood of paternity. Log-likelihoods are relative, not absolute, measures of paternity. In general, only the difference between two males in log-likelihood of paternity can be interpreted. However, a log-likelihood of $-\infty$ has a clear interpretation: the male is excluded from paternity. To make inferences about paternity, we used the difference in log-likelihood of paternity (symbolized by ΔL) between the colony male(s) with highest L and the coterie male with highest L. If the coterie male with highest L is also the colony male with highest L, then ΔL equals 0. In all other instances, ΔL is positive. As used here, ΔL is similar to the likelihood ratio or G test of Sokal and Rohlf (1969). However, there are no degrees of freedom associated with ΔL, and no comparison with the Chi-square or other distribution is possible. Edwards (1972) used a log-likelihood difference of 2 as a critical level to make inferences. With 52 values of ΔL to be interpreted, however, the use of Edwards' criterion would result in an unacceptably high risk of falsely rejecting the null hypothesis that each female was inseminated by a coterie male (type I error). Therefore, we used the more conservative value of 4 as the critical level. For each litter, we calculated L for each adult male in the study colony. If ΔL was less than 4, we accepted the null hypothesis that the female was inseminated by a male in her coterie. If ΔL was greater than 4, we rejected the null hypothesis and concluded that the female was inseminated by a male other than one in the coterie. An example of our statistical methods is provided in Table 1.

RESULTS

Four proteins were electrophoretically polymorphic in the study colony: transferrin, esterase, nucleoside phosphorylase, and 6-phosphogluconate dehydrogenase (the lat-

TABLE 1.—*Example showing genotypes and calculation of log-likelihood of paternity for one black-tailed prairie dog litter born in 1980 in the Wind Cave colony, South Dakota. The difference in log-likelihood between the colony male with highest likelihood of paternity (male 23) and the coterie male with highest likelihood of paternity (male 22) is 0.69, so the hypothesis that the coterie male inseminated the female was not rejected in this example.*

Locus	Males		Female	Offspring	
	22	23	46	1	2
Transferrin	BC	CD	BB	BC	BC
Esterase	AB	AA	AB	AA	AB
Nucleoside phosphorylase	BB	BC	BC	BB	BB
6-Phosphogluconate dehydrogenase	AB	BB	AB	BB	BB

	Likelihood of paternity for male 22	Likelihood of paternity for male 23
Transferrin	$\frac{2!}{2!}(0.5)^2 = .25$	$\frac{2!}{2!}(0.5)^2 = 0.25$
Esterase	$\frac{2!}{1!\,1!}(0.25)^1(0.5)^1 = 0.25$	$\frac{2!}{1!\,1!}(0.5)^1(0.5)^1 = 0.5$
Nucleoside phosphorylase	$\frac{2!}{2!}(0.5)^2 = 0.25$	$\frac{2!}{2!}(0.25)^2 = 0.0625$
6-Phosphogluconate dehydrogenase	$\frac{2!}{2!}(0.25)^2 = 0.0625$	$\frac{2!}{2!}(0.5)^2 = 0.25$
Combined	$(0.25)(0.25)(0.25)(0.0625) = 0.000977$	$(0.25)(0.5)(0.0625)(0.25) = 0.001953$
Log-likelihood of paternity		
Male 22		-6.93
Male 23		-6.24
All other adult males (not shown)		$-\infty$

ter not scored in 1979 samples). The transferrin and nucleoside phosphorylase polymorphisms each consisted of six phenotypes (AA, AB, BB, AC, BC, and CC) interpreted to be controlled by three codominant alleles (A, B, and C) segregating at one autosomal locus. The esterase and 6-phosphogluconate dehydrogenase polymorphisms each consisted of three phenotypes (AA, AB, and BB) interpreted to be controlled by two codominant alleles (A and B) segregating at one autosomal locus. For each protein, all phenotypes predicted by the respective genetic model were observed in the study colony. For all proteins, each offspring shared at least one electromorph with its mother, as expected for proteins controlled by codominant alleles.

In 1979, 31 adult males (numbered 0–30) resided in the colony and 22 females (numbered 1–22) produced litters (Table 2). Females 17 through 22 produced litters again in 1980, and 24 more litters were produced in 1980 by females 23 through 46. Males 17 through 30 survived until 1980. Also, 13 new adult males (numbered 31–43) were resident in the colony in 1980 (Table 3); most of these were yearlings in the colony in 1979. For 32 of 52 litters, at least one of the males in the coterie was included in the set of males with highest likelihood of paternity. For the remaining 20 litters, none of the coterie males had the highest likelihood of paternity when compared with all other adult males resident in the colony. Because this circumstance could easily arise by chance when only a few polymorphic loci were studied, it was important to determine whether a male other than one in the coterie had a significantly greater likelihood of paternity. For 18 of 20 litters, the value of ΔL was less than 4, so we concluded that these females were inseminated by coterie males. Only for litters born

TABLE 2.—*Log-likelihood of paternity (L), based on three polymorphic loci, for 22 black-tailed prairie dog litters born in 1979 in the Wind Cave colony, South Dakota. ΔL is the difference in log-likelihood between the colony male(s) with highest likelihood of paternity and the coterie male with highest likelihood of paternity.*

Female	Coterie male(s)	L	Male(s) with highest L	ΔL
1	23	−1.96	23	0
2	23	−4.45	23	0
4	9	−2.08	9	0
6	15	−4.16	15, 26	0
	29	−∞		
7	24	−4.16	24	0
	25	−8.32		
8	24	−0.98	24	0
	25	−5.14		
11	28	−4.73	4, 28	0
14	16	−4.92	0, 4, 16, 18, 25, 27, 28	0
17	13	−0.69	13, 29	0
18	10	−1.96	10	0
	11	−∞		
19	23	−4.85	23	0
22	23	−4.16	23	0
9	26	−2.08	5, 7	1.39
12	26	−4.73	13, 29	1.39
13	27	−5.43	0, 16, 18	1.39
15	14	−2.08	24	1.39
16	27	−3.47	2, 6, 17, 20, 24	1.39
20	27	−2.08	8, 12	1.39
3	30	−5.14	19	2.08
21	9	−3.06	8, 12	2.08
5	12	−∞	23	∞
10	12	−∞	11, 21, 30	∞

to females 5 and 10 in 1979 was there a significantly greater likelihood of paternity for a male other than one of the coterie males. Both instances involved male 12, and for both litters this male was excluded from paternity ($L = -\infty$) by the data for transferrin and nucleoside phosphorylase. Because paternity exclusions were observed for two loci simultaneously, it is unlikely that these results were caused by new mutations in the offspring or null (electrophoretically undetectable) alleles in male 12. For both transferrin and nucleoside phosphorylase, the single offspring of female 10 was heterozygous for an allele not present in either the mother or male 12, which excluded the null-allele explanation. Therefore, we concluded that females 5 and 10 were inseminated by males other than the resident coterie male.

Between 1979 and 1980, 18 litters were born to females living in multi-male coteries, and for 12 of these all but one of the coterie males were excluded from paternity (1979—litters of females 6 and 18; 1980—litters of females 17, 18, 19, 22, 24, 25, 33, 36, 38, and 41). For the litters of females 7 and 8, both coterie males were possible fathers; however, in both instances the difference between the two males in log-likelihood of paternity was 4.16, so paternity could be assigned to one of the males (male 24). For the remaining four litters (those of females 34, 35, 37, and 39), the difference between the two possible fathers in log-likelihood of paternity was too small to permit assignment of paternity. In at least three of the five multi-male coteries for which several females produced litters, a single coterie male was responsible for all inseminations. The most exact inference about paternity was made when all but one male in the study colony were excluded, a result obtained for 13 of the 52 litters (1979—litters of females 1, 2, 4, 5, 18, 19, and 22; 1980—litters of females 18, 21, 29,

TABLE 3.—*Log-likelihood of paternity, based on four polymorphic loci, for 30 black-tailed prairie dog litters born in 1980 in the Wind Cave colony, South Dakota. Symbols as in Table 2.*

Female	Coterie male(s)	L	Male(s) with highest L	ΔL
17	38	−2.08	25, 27, 38	0
	26, 39	−∞		
18	42	−2.77	42	0
	41	−∞		
20	37	−0.69	17, 20, 37	0
21	22	−2.94	22	0
23	27	−5.43	25, 27, 38	0
24	28	−4.69	28	0
	31	−∞		
25	32	−0.98	32	0
	33	−∞		
26	35	−3.06	31, 35, 40	0
27	35	−3.06	35, 40	0
29	30	−4.73	30	0
30	30	−6.81	30, 43	0
33	38	−2.94	25, 27, 38	0
	26, 39	−∞		
34	26	−2.77	26, 29, 35, 40, 43	0
	39	−3.47		
	38	−∞		
36	19	−4.04	19	0
	20	−∞		
38	19	−5.14	19	0
	20	−∞		
39	19	−2.08	19, 31	0
	20	−2.77		
40	25	−2.94	25, 27, 38	0
41	42	−2.08	42	0
	41	−∞		
43	43	−4.88	43	0
45	22	−3.47	22	0
31	37	−2.66	31	0.69
32	17	−4.16	31	0.69
35	26	−8.20	41	0.69
	38	−10.97		
	39	−∞		
37	19	−4.16	31	0.69
	20	−4.16		
42	24	−1.39	31, 41	0.69
46	22	−6.93	23	0.69
19	18	−4.16	24	1.39
	40	−∞		
22	18	−5.43	24, 34, 36	1.39
	40	−∞		
44	22	−3.47	18, 24, 29, 33	1.39
28	36	−4.73	31	2.77

41, 43, and 45). In addition, for litters of two females (25 and 36) all but one male were eliminated by use of a critical value of 4.

DISCUSSION

This study demonstrates that there is a close correspondence between the social structure of the black-tailed prairie dog as determined by observation and the mating

system as revealed by electrophoretic analysis. That is, a female usually mates with one of the resident males in her coterie. There were two exceptions, however: male 12 could not have fathered the two litters born in his coterie in 1979. Also, the genetic analysis provided information that would be difficult or impossible to obtain by observation. In particular, for 14 of 18 litters (78%) born in multi-male coteries, paternity was unambiguously assigned to one of the coterie males.

The five assumptions of our method were tested retrospectively. First, the mother-offspring comparisons supported the assumption that each polymorphism was controlled by codominant alleles at a single autosomal locus. Second, an analysis of the progeny born to homozygous females and segregating for two paternal alleles revealed a close fit to the expected 50:50 ratio. An analysis of progeny born to females doubly heterozygous for any of the six possible two-locus combinations indicated that the hypothesis of tight linkage (recombination fraction 0.05 or less) could be rejected in five. There was no information available about possible linkage between transferrin and 6-phosphogluconate dehydrogenase. Therefore, the assumptions of Mendelian segregation and independent assortment were met at least approximately. Third, transient males were never seen at the study site during the breeding season, and for no litter were all males in the colony excluded from paternity. These observations support the assumption that adult males resident in the colony were responsible for all inseminations. Fourth, the assumption that we knew the genotype at all loci for each male in the colony was violated only once: a male that died after the 1980 mating season before blood samples were taken. Fifth, there was no evidence for multiple insemination of females in the form of transmission to progeny in the same litter of three different paternal alleles for transferrin or nucleoside phosphorylase.

A limitation of our method is that homozygous males have a higher likelihood of paternity than heterozygous males for most litters for which homozygotes are not excluded from paternity. For example, the relatively high frequency with which male 31 occurred in the sets of males with highest likelihood of paternity in 1980 does not necessarily indicate a high level of sexual activity. Instead, this result probably reflects the fact that male 31 was homozygous for the most common allele at all four loci. To some extent, "bias" in favor of homozygous males in the likelihood analysis is balanced by the greater chance that they will be excluded from paternity, compared with heterozygous males. For a two-allele locus, of course, a heterozygous male can never be excluded from paternity. A second limitation of the method is that the strength of the likelihood analysis depends on the number of polymorphic loci examined and on the litter size, being more powerful with more loci and larger litters. Both behavioral and genetic data clearly are needed to provide a complete description of the mating system in the black-tailed prairie dog. However, for 29% of the litters it was possible to eliminate all but one male as the father. By studying several additional polymorphisms, it should be possible to increase the number of males excluded from paternity and thus approach an entirely genetic description of the mating system in black-tailed prairie dogs.

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