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Steven E. Travis
Northern Arizona University

C. N. Slobodchikoff
Northern Arizona University

Paul Keim
Northern Arizona University

DNA FINGERPRINTING REVEALS LOW GENETIC DIVERSITY IN GUNNISON'S PRAIRIE DOG (*CYNOMYS GUNNISONI*)

Steven E. Travis, C. N. Slobodchikoff, and Paul Keim

*Department of Biological Sciences, Box 5640,
Northern Arizona University, Flagstaff, AZ 86011-5640*

The use of molecular techniques for the assessment of familial relationships among social species of mammals has become relatively commonplace. However, some species represent poor candidates for such studies due to naturally low levels of genetic diversity, leading to unacceptably large standard errors associated with estimates of relatedness. Here, we report on a preliminary study of genetic diversity within two populations of a social species of ground squirrel, Gunnison's prairie dog (*Cynomys gunnisoni*) using DNA fingerprinting. We observed low levels of diversity in the form of large mean coefficients of genetic similarity among individuals occupying the same population. Overall similarity, determined from the combined data, yielded by three minisatellite probes, ranged from 55 to 61%. These values place Gunnison's prairie dog at the extreme upper end of the range of similarity values reported for outbred species of mammals (ca. 0.20-0.50). As a partial means of explaining these results, and as a means of comparing our results to those of similar studies using allozymes, we determined the level of differentiation between our two study colonies in the form of an F-statistic analog. A value of 0.11 ($\pm$ 2.26 $\times$ 10^{-3}) was obtained and is similar to values reported from allozyme studies (0.07-0.12). A significance test of this value yielded a positive result (D = 5.63, $d.f.$ = 1, P < 0.025), demonstrating that gene flow between populations is limited, a factor that may help to maintain low levels of diversity.

Key words: *Cynomys gunnisoni*, genetic diversity, population differentiation, DNA fingerprinting

Traditionally, behavioral and population ecological studies requiring a knowledge of familial relationships within and among social groups have had to rely on the construction of pedigrees from observational data (Clutton-Brock, 1989). Early attempts to overcome the time-consuming nature and potential biases associated with this approach made use of allozyme polymorphisms (Hanken and Sherman, 1981; Hoogland and Foltz, 1982; Schwartz and Armitage, 1980; 1981). However, average heterozygosities and the proportion of loci that are actually polymorphic tend to be quite low for most species of mammals (Nevo, 1978). Because determinations of genetic relatedness often depend on the experimenters ability to unequivocally assign par-

entage to individuals, low levels of genetic heterogeneity may prove highly problematic (Lynch, 1988). As a result, there has been a concerted effort over the past decade to develop molecular techniques for the rapid generation of hypervariable genetic markers (Jeffreys et al., 1985*a*; Tautz, 1989; Vos et al., 1995; Williams et al., 1990). Improved results have been obtained from RFLP analyses (Amos et al., 1991; Harris et al., 1991; Inoue et al., 1991; Jeffreys et al., 1985*b*; Lehman et al., 1992; Packer et al., 1991; Paul et al., 1992*a*, 1992*b*; Ribble, 1991; Tegelstrom et al., 1991), particularly those involving minisatellite probes used to construct DNA fingerprints.

The accurate assignment of genetic relationships from molecular data has some dif-

ficulties. Despite high levels of genetic heterogeneity apparent from DNA fingerprints of most mammalian species (Gilbert et al., 1991; Hoagland et al., 1991; Ribble, 1991), there are, nevertheless, highly inbred species for which genetic profiles remain nearly identical among individuals, particularly when related individuals are included (Gilbert et al., 1990*a*; Reeve et al., 1990). Under such circumstances, the mean genetic similarity for related individuals of a given order may be represented by an unacceptably large standard error for the assignment of a specific relationship (Lynch, 1988). Therefore, it may not be possible to assign levels of relatedness by previously established methods, such as through the use of a calibration curve (Gilbert et al., 1991), but rather may require the formulation of an alternative method (Travis et al., 1996). A preliminary assessment of overall similarity among the members of a population or species must necessarily be conducted prior to more in-depth studies of relatedness (Lynch, 1988). A preliminary study of this kind may provide an added benefit by yielding the data necessary for a further assessment of population structure. In this way, clues as to how the existing level of genetic heterogeneity is maintained may be provided, an issue that may be of potential consequence to the social dynamics occurring within and among groups of relatives.

This report presents the preliminary findings from a much broader study of dynamics of social systems in prairie dogs, using DNA fingerprinting as a means of assessing genetic relatedness (Travis et al., 1995, 1996). Specifically, we studied Gunnison's prairie dog (*Cynomys gunnisoni*), whose range is restricted to grasslands of the Colorado Plateau encompassing portions of Arizona, New Mexico, Utah, Colorado, and Wyoming (Hall, 1981). Like other members of the genus *Cynomys*, such as the more intensively studied black-tailed prairie dog (*C. ludovicianus*; Hoogland, 1981*a*, 1981*b*, 1982, 1983, 1986, 1992; Hoogland and Foltz, 1982), Gunnison's prairie dogs were once characterized by vast colonies of individuals. Cattle grazing, as well as land development, have both contributed to the relatively recent fragmentation of many colonies (Koford, 1958), potentially lowering population genetic heterogeneity by creating isolated demes, which may further be subject to the effects of random genetic drift. Therefore, one might expect high overall levels of similarity among DNA fingerprints representing prairie dogs collected within the same sites, as well as significant levels of differentiation among sites. Allozyme evidence exists to support this hypothesis (Chesser, 1983; Daley, 1992; McCullough and Chesser, 1987), and it remains to be seen whether DNA-fingerprinting data will corroborate previous results.

Therefore, our purpose was twofold. First, we set out to establish the overall level of genetic heterogeneity at two sites for Gunnison's prairie dogs where our research on the social system has been ongoing since 1987 (Travis and Slobodchikoff, 1993; Travis et al., 1995, 1996). Second, we attempted to explain partially the observed levels of heterogeneity through an assessment of population structure, and to determine the reliability of DNA fingerprinting for this purpose by comparing our results to those of other researchers who have been concerned with this issue as it pertains to social species of ground squirrels.

MATERIALS AND METHODS

Two colonies of Gunnison's prairie dogs near Flagstaff, Arizona, described previously as Antelope Hill and Potato Lake (Travis and Slobodchikoff, 1993; Travis et al., 1995, 1996), were characterized and compared genetically by DNA fingerprinting. On the basis of known densities within the study areas, each colony was estimated to consist of several thousand individuals. Spatial separation between study colonies was ca. 13 km, a distance exceeding the maximum dispersal distance reported for prairie dogs, or 10 km (Knowles, 1985). However, the existence of several intervening colonies (not characterized) between 2- and 5-km distances from the colonies of interest should have allowed gene

flow to occur through a series of intermediaries. Habitat lying between colonies consisted primarily of ponderosa pine (*Pinus ponderosa*) forest.

Intensive live-trapping, marking, and tissue-sampling procedures were conducted on 1.44-ha study plots at each colony, one to two times per week throughout the active season (early April through late October) of 1991. These areas represented a small subregion within each colony, encompassing five to ten group territories (Travis et al., 1996). Trapping was highly successful and resulted in the collection of all adults from the study plots, as confirmed by observations of marked animals from elevated viewing towers erected at each colony. Age was assessed on the basis of trapping records compiled over a 5-year period beginning in 1988, and, where necessary, from mass measurements taken by placing individuals in a cloth sac suspended from a spring scale. Blood samples were collected from all adult animals captured by toenail clipping from a hind foot. Blood was rinsed into 15-ml, polypropylene, centrifuge tubes using an isotonic saline buffer (1X SSC: 0.15 M NaCl, 15 mM sodium citrate, 1 mM EDTA). Samples were stored on ice in the field, and subsequently frozen at −70°C.

Total genomic DNA was extracted from blood samples according to the procedures of Müllenbach et al. (1989). The amount of DNA was quantified via fluorometry and adjusted to a final concentration of 150 μg/ml. Four μg of DNA from each sample was digested with 16 units of *Hae* III in the presence of 1 mM spermidine, for 5 h at 37°C. Digested samples were loaded on 0.7% agarose gels as randomized alternating pairs of adult individuals from each colony according to the recommendations of Lynch (1990). In addition, because comparisons of DNA fingerprints were to be conducted across several gels, each gel was loaded with two to six standards representing prairie dogs killed from outside the study plot at Antelope Hill. Because the random sharing of fragments was high in this species (see below), all fragments scorable among members of the two study populations were present in these standards, providing a means of aligning fragments across multiple gels. DNA fragments were electrophoresed on 20 by 22-cm gels at 2.2 V/cm for 24 h and transferred to Amersham Hybond N+ membranes by southern blotting in 0.40 N NaOH. Membranes were hybridized sequentially with three minisatellite probes; pV47-2 (Longmire et al., 1990), 33.15, and 33.6 (Jeffreys et al., 1985a). Probe DNA (10–25 ng) was labeled with ^{32}P-dCTP by the random primer method (Feinberg and Vogelstein, 1983). Prehybridization, hybridization, and washes were conducted according to Gilbert et al. (1990b). Membranes were autoradiographed at −70°C for 1-14 days using Konica Medical X-Ray Film and intensifying screens. Prior to reprobing, DNA probes were stripped from membranes by washing in 0.5 N NaOH, 0.1% SDS and 0.25 M Tris-HCl (pH 7.0).

We determined mean coefficients of similarity, S (Lynch, 1988), from profiles of individual DNA fingerprints compared among adult individuals both within and between colonies. This coefficient is calculated as the proportion of bands present in a given pair of individuals that are shared (i.e., $S = 2N/(N_A + N_B)$, where N is the number of bands shared between individuals A and B, and N_A and N_B are the total number of bands in individual A and individual B, respectively). Based on values of S, we calculated an analog of Wright's F_{ST}, denoted as F' (Lynch, 1990), as a means of determining the level of genetic differentiation between colonies. Mean similarity coefficients were calculated from all possible pairwise comparisons of individuals and for all probes both individually and combined. Before data from separate probes could be combined, however, it was necessary to determine whether any fingerprinting bands were revealed simultaneously by more than one probe, thereby yielding redundant information. We tested for nonindependence of bands by comparing similarly sized bands (determined from molecular weight ladders) among all individual prairie dogs across all probes. Nonindependence was concluded when identical banding patterns were observed across probes in 90% (chosen arbitrarily) of the individuals investigated. No instance of nonindependence was detected in these data, and combined counts of alleles detected from separate probes was considered justified.

Scoring of DNA fingerprints was conservative. All bands apparent from each individual fingerprint were first ranked for their relative intensity within each lane as either low, medium, or high. Only medium and high intensity bands were used to compare across individuals. Shared bands were then assigned strictly on the basis of

TABLE 1.—*Band-sharing statistics from comparisons of DNA fingerprints constructed using three minisatellite probes; pV47-2, 33.15, and 33.6. DNA fingerprints from Gunnison's prairie dogs for two colonies in Arizona; Antelope Hill (AH) and Potato Lake (PL).*

| Probe | Comparisons | | Scorable bands per individual | | | Coefficients of similarity | | |
	Type	Number	Mean	Range	SD	Mean	Range	SD
pV47-2	AH (within)	780				0.62	0.24–0.95	1.60×10^{-4}
	PL (within)	861				0.67	0.33–0.94	5.60×10^{-4}
	AH/PL (between)	1,680	9.29	6–14	3.74	0.58	0.25–0.91	9.20×10^{-5}
33.15	AH (within)	820				0.51	0.00–0.94	2.14×10^{-4}
	PL (within)	861				0.58	0.00–0.94	2.90×10^{-4}
	AH/PL (between)	1,722	9.55	5–14	3.57	0.51	0.11–0.87	3.80×10^{-5}
33.6	AH (within)	820				0.46	0.00–1.00	NA[a]
	PL (within)	861				0.49	0.00–1.00	NA[a]
	AH/PL (between)	1,722	3.57	2–7	1.60	0.41	0.00–1.00	NA[a]
Combined	AH (within)	820				0.55	0.22–0.84	9.10×10^{-6}
	PL (within)	861				0.61	0.30–0.87	2.17×10^{-4}
	AH/PL (between)	1,722	22.16	15–30	12.97	0.53	0.22–0.79	1.51×10^{-4}

[a] Sampling variance was not calculated due to a severely limited number of scorable bands.

size, without regard for intensity of band. Because we eliminated many low-intensity bands by this technique, the mean number of bands scorable per individual was <10 for each of the three probes. For this reason, a value of F' was calculated only on the basis of data from all probes combined.

Lynch (1990) provided the means of calculating and testing for significance of an analog of Wright's F_{ST} from DNA fingerprint data calculated as: $F' = (1 - S_b)/(2 - S_w - S_b)$, where S_b is the mean coefficient of similarity between members of separate populations, and S_w is the mean coefficient of similarity within populations averaged over all colonies. Lynch and Crease (1990) provided a formula for calculating the sampling variance of F', that is obtained by a first-order Taylor expansion. This variance statistic accounts for the covariance arising from multiple comparisons among a relatively small group of individuals. The test statistic, D, was used to test the null hypothesis of no population subdivision assuming a normal distribution of F', where $D = F'^2/\text{Var}(F')$. D is distributed as a chi-square with one degree of freedom (Lynch and Crease, 1990). Our calculation of F' did not account for the tendency of prairie dogs to form distinct lineages within group territories (Chesser, 1983), which may lead to localized differentiation within colonies and inflate variance estimates (Chesser, 1991). Therefore, our significance test should be considered conservative.

RESULTS

High levels of genotypic identity-in-state were detected among adult individuals within colonies, as revealed by unusually high mean within-colony coefficients of similarity (Table 1). Mean coefficients of similarity were somewhat lower at Antelope Hill, where they were based on 820 pairwise comparisons of 41 individuals (range, 0.46–0.62 among the three probes scored individually), than at Potato Lake, where they were based on 861 pairwise comparisons of 42 individuals (range, 0.49–0.67). These differences were reflected in the mean coefficients of similarity for all probes combined, with Antelope Hill showing a value of 0.55, and Potato Lake showing a value of 0.61. As expected, mean coefficients of similarity calculated on the basis of between-colony pairwise comparisons ($n = 1,722$) were consistently lower than those representing within-colony comparisons, ranging from 0.41 to 0.58 among individual probes. The between-colony coefficient of similarity for all probes combined was 0.53. Sampling variances are given in Table 1.

Our results indicate a significant level of genetic differentiation between the Ante-

lope Hill and Potato Lake colony ($D = 5.63$, *d.f.* $= 1$, $P < 0.025$), with an F'-value of 0.11 for all probes combined and a sampling variance of 2.26×10^{-3}. If it is assumed that F_{ST} will remain constant over time, due to a balance between genetic drift and gene flow, then the number of dispersers moving between colonies each generation can be estimated from the following formula: $N_e m = (\frac{1}{4}F_{ST}) - \frac{1}{4}$, where N_e is the effective population size and m is the migration rate (Wright, 1969). Using this formula in concert with an F_{ST} of 0.11, we obtain a value of 2.02 dispersers per generation necessary to maintain the current level of population differentiation over 13 km. It is unlikely that dispersal between colonies occurred in a single step due to the limited dispersal capabilities of prairie dogs; therefore, the level of gene flow between nearest-neighboring colonies would have to be >2.02 dispersers to maintain the observed level of gene flow.

DISCUSSION

The similarity coefficients revealed here are higher than those generally reported for other outbred mammalian species. The lowest values reported for outbred species are at or just below 0.20 (e.g., 0.16 for the California mouse, *Peromyscus californicus*, using probe 33.6—Ribble, 1991), while the highest values rarely exceed 0.50 (e.g., 0.49 for the African lion, *Panthera leo*, using a feline-specific minisatellite probe—Gilbert et al., 1991, Packer et al., 1991). Highly inbred species may display similarity coefficients approaching unity, as is exemplified by the naked mole rat (*Heterocephalus glaber*), where S-values as high as 0.99 have been revealed using the probe M13 (Reeve et al., 1990). It appears that Gunnison's prairie dogs fall at the upper end of the range of S-values representing outbred mammals. This is perhaps not surprising because an earlier study by Benedix (1988) reported complete monomorphism at 38 of 40 allozyme loci investigated, although no estimate of F_{ST} was calculated.

Frequent population bottlenecks resulting from a recent history of outbreaks of sylvatic plague may have contributed to the paucity of genetic diversity within populations of Gunnison's prairie dog. The prevalence of the plague-causing bacterium, *Yersinia pestis*, among populations of all five North American species of prairie dogs is well-documented. Rates of mortality during plague epizootics have been known to exceed 97%, often nearly eliminating entire colonies within a single active season (Barnes, 1982; Cully, 1989, 1991; Lechleitner et al., 1962, 1968; Olsen, 1981; Rayor, 1985). For example, Rayor (1985) reported the complete elimination of a colony of Gunnison's prairie dogs numbering 1,000–1,500 individuals within a period of 2 months, while Cully (1991), working with the same species, reported a reduction in size of colony from 2,800 to 120 individuals over one active season. Such extreme bottlenecks may contribute to the temporal persistence of pronounced founder effects and subsequent drift, which ultimately may contribute to genetic differentiation among populations.

Restricted gene flow, as evidenced by a significant level of differentiation between colonies, may further have contributed to the maintenance of low levels of diversity within populations. Possible barriers to gene flow between colonies existed in the form of intervening forest habitats that may have been inhospitable to dispersing prairie dogs. The existence of territoriality within colonies may have further hindered dispersal by subjecting individuals to high levels of aggression as they attempted to undertake long-range movements (Chesser, 1983; Rayor, 1988).

The persistence of kin groups of females within shared territories, a characteristic of prairie dogs as well as many other species of North American ground squirrels (Anderson, 1989), is of potential consequence to the maintenance of genetic homogeneity within colonies and genetic differentiation between colonies, because it favors high

levels of coancestry within territorial social groups (Chesser, 1991). For example, the persistence of a female on a territory occupied by several different breeding males over several consecutive breeding seasons may produce multiple litters of half-sibs. Alternatively, coancestry within territories would be promoted in situations where males maintain their associations with specific kin groups of females for a period of several consecutive years. This could allow sufficient time for the attainment of reproductive maturity by their female offspring, with whom they then could mate.

The level of genetic differentiation reported in the current investigation is represented by an analog F_{ST}-value that is equal to or slightly higher than those reported in other studies of population subdivision among species of social North American ground squirrels. The majority of these studies report overall values of F_{ST} closely approximating 0.07. Schwartz and Armitage (1980) reported a mean F_{ST} of 0.07 using eight variable allozyme systems to estimate differentiation among nine populations of the yellow-bellied marmot (*Marmota flaviventris*). Chesser (1983) reported a range of F_{ST}-values with a mean of 0.07 among populations of the black-tailed prairie dog occupying the same geographic region, using seven variable allozyme systems. Finally, McCullough and Chesser (1987) reported an overall F_{ST}-value of 0.07 among three populations of the Mexican prairie dog (*C. mexicanus*) based on electrophoresis of 30 enzyme loci. Daley (1992) provided the sole exception, reporting a mean F_{ST} of 0.12 among eight culled populations of the black-tailed prairie dog using four variable allozyme systems. Thus, it is clear that the analog F_{ST}-value of 0.11 reported for the Gunnison's prairie dog using DNA fingerprinting is similar to values reported using allozyme systems. The slight differences that do exist are not likely to be statistically (or biologically) significant due to a relatively large standard error associated with our reported value, i.e., 0.0475.

In conclusion, our results suggest a need to exercise caution in the application of DNA fingerprinting data to studies of genetic relatedness in Gunnison's prairie dog, where the assignment of specific relationships is called for. Observed coefficients of similarity in excess of 0.60 necessarily must have been due to small numbers of alleles representative of the minisatellite loci screened by the three probes used in this study. Likewise, the overall number of loci detected by DNA fingerprinting was relatively small, something substantially <30 (determined simply on the basis of the maximum number of fragments scorable for any one individual in our study). Lynch (1988) has determined that, even in highly diverse species with an extreme of 100 alleles per minisatellite locus, the standard errors of estimate for first-, second-, third-, and fourth-order relatives, given information on 25 loci, are 14, 20, 35, and 53%, respectively. For Gunnison's prairie dogs, it would be necessary to allow for a much larger margin of error that would all but eliminate the possibility of establishing specific relationships among individuals of interest. Nevertheless, the estimation of comparative levels of relatedness remain possible, allowing for the assessment of differences in overall levels of relatedness among experimental groups (Travis et al., 1995). In addition, high levels of DNA-fingerprint similarity do not preclude the assignment of parent-offspring relationships by paternity testing (Travis et al., 1996). Thus, we have shown that, while the utility of DNA fingerprinting to studies of mammalian social systems may be limited in species lacking genetic diversity such as Gunnison's prairie dog, as long as a conservative approach to the assessment of relatedness is exercised, molecular techniques such as this may yield information that would not be available by any other means.

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