KINSHIP, SOCIAL RELATIONSHIPS, AND DEN SHARING IN KIT FOXES

KATHERINE RALLS,* KRISTINE L. PILGRIM, P. J. WHITE, ELENI E. PAXINOS, MICHAEL K. SCHWARTZ, AND ROBERT C. FLEISCHER


We used 11 microsatellites, highly variable nuclear markers, to infer kinship among 35 San Joaquin kit foxes, Vulpes macrotis mutica, and combined this information with field observations to gain insight into fox social behavior. Fox social units consisted of solitary foxes, mated male–female pairs, and trios consisting of a mated pair plus another adult. Pair-mates were not closely related. The additional adult (1 male, 1 female) in 2 trios was the offspring of at least 1 of the pair-mates. Foxes living on adjacent home ranges tended to be more closely related than foxes that did not live on adjacent home ranges, largely because females on adjacent home ranges were often closely related. F_is values indicated a deficiency of homozygotes that was likely due to clusters of relatives living on adjacent home ranges. Foxes that shared the same den on the same day were usually members of the same social group. Contrary to expectations, however, we sometimes found foxes sharing dens with foxes from other social groups. Many cases involved unpaired individuals and appeared to be unsuccessful attempts at pair formation. Other cases involved members of 2 adjacent social groups, a pair and a trio. Both members of the pair were closely related to ≥1 member of the trio, indicating that kit foxes can maintain enduring social relationships with adult offspring or siblings that have dispersed to a new home range and found a mate.

Key words: dens, DNA, kinship, mating systems, microsatellites, relatedness, San Joaquin kit fox, social organization, Vulpes macrotis mutica

Kinship influences behavior in many species of vertebrates. In general, close kin avoid mating with each other but tend to associate and cooperate more than unrelated individuals (Emlen 1997). Thus, information on kinship among individuals can greatly facilitate understanding of their behavior toward one another. The ability of biologists to infer the degree of kinship among individuals in wild populations has been much improved by development of new classes of genetic markers, such as microsatellites (Queller et al. 1993), and associated analytical techniques for calculating kinship based on these highly variable nuclear markers (Goodnight and Queller 1999; Queller and Goodnight 1989). For example, microsatellite analyses have shown that incestuous pairings are rare in gray wolves (Canis lupus—Smith et al. 1997) and African wild dogs (Lycaon pictus—Girman et al. 1997).

Like other small canids, kit foxes (Vulpes macrotis) are thought to be monogamous (Geffen et al. 1996). Pups are born once a year in February or March, and the majority die or leave their parents’ home ranges before the next breeding season (Koopman et al. 2000). Many juvenile kit foxes either exhibit natal philopatry or disperse for rela-
tively short distances (Scrivner et al. 1987). The occasional extra adult associated with a mated pair (Egosue 1962; O’Neal et al. 1987; White and Ralls 1993) is thought to be 1 of that pair’s grown pups from the previous year that has not yet dispersed (Geffen et al. 1996; Moehlman 1986; Moehlman and Hofer 1996), although this assumption has not been tested with molecular genetic markers.

Kit foxes escape from high temperatures of their desert environment and their predators by spending the day in an underground den (Golightly and Ohmart 1984; Seton 1925). Individual foxes typically remain in the same den all day, emerge at night to hunt, and return to the same den or a different den the next morning. Social groups of kit foxes maintain numerous dens in relatively exclusive denning ranges that overlap only slightly with the denning ranges of adjacent groups (K. Ralls and P. J. White, in litt.; Spiegel 1996). Members of the same social group often share the same den, with mated males and females found in the same den on about 45% of the days both individuals are located via radiotelemetry (Koopman et al. 1998).

We investigated the relationship between kinship and several aspects of behavior of kit foxes. We gathered data on social relationships, home ranges, and use of dens of San Joaquin kit foxes (V. m. mutica) in the Carrizo Plain Natural Area, California, by tracking radiocollared foxes and used microsatellites to infer kinship among these foxes. We predicted that mated pairs would not be closely related (Emlen 1997; Ralls et al. 1986; Smith et al. 1997) and that an additional adult using the same home range as a mated pair would be an offspring of that pair (Geffen et al. 1996; Moehlman 1986; Moehlman and Hofer 1996). Because natal philopatry and short-range dispersal are common in kit foxes (Scrivner et al. 1987; Waser and Jones 1983), we predicted that foxes on adjacent home ranges would be more closely related than foxes that did not live on adjacent home ranges. Finally, we predicted that an adult kit fox would share the same den on the same day only with foxes in the same social group, that is, its mate and their juvenile or adult offspring (Koopman et al. 1998).

**Materials and Methods**

*Study area.*—The study was conducted in the western part of the Carrizo Plain Natural Area (39°15’N, 119°W), San Luis Obispo County, California (White and Ralls 1993:865, figure 1). The study area ranged in size from 85 km² in 1989 to 140 km² in 1991. The principal habitat types within the study area were valley grassland, alkali sink, and fallow grain fields. Detailed descriptions of the vegetation types, climate, and fauna are provided in White and Ralls (1993). Nocturnal rodents were the principal prey of the foxes (White et al. 1996). Average annual precipitation in the study area was 26 cm, occurring primarily as winter rains. However, the study was conducted during a drought that reduced populations of small mammals, causing greatly reduced reproductive success in the kit foxes (White and Ralls 1993). Hence, we were able to obtain DNA samples from only 3 pups.

Larger canids, particularly coyotes (*Canis latrans*), killed about half the adult foxes on the study area each year (Ralls and White 1995), which resulted in frequent changes in fox social groups. However, a few foxes survived throughout the study.

*Determining social relationships, den sharing, and neighboring foxes.*—From December 1988 through November 1990, we captured kit foxes in Tomahawk wire box traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin) baited with sardines. Each captured fox was examined for sex, weighed, and fitted with a radiocollar weighing about 45 g. Five to 10 cc of blood were drawn from the femoral vein or carotid artery of each fox and immediately placed in a 1-cc ethylenediaminetetraacetic acid disodium salt (EDTA) tube. Samples were refrigerated as soon as possible and mailed overnight in a styrofoam container to the laboratory for DNA extraction. When catching and handling foxes, we followed a United States Fish and Wildlife animal-welfare protocol for the endangered San Joaquin kit fox. We routinely recaptured foxes and replaced their collars.

We monitored radiocollared individuals close-
ly to identify mated pairs, extra adults associated with mated pairs, individuals that shared dens, and foxes that lived on adjacent home ranges. We tracked each radiocollared fox to its den each day using a handheld antenna. We also located foxes at night to determine home ranges. Details on radiotelemetry techniques, methods of estimating home ranges, and maps of convex polygon and harmonic mean home ranges within the study area are given in White and Ralls (1993) and White et al. (1994). We considered that 2 adult foxes of opposite sex were a mated pair if they had similar home ranges and they frequently and concurrently shared the same den (Koopman et al. 1998; White and Ralls 1993). When a social group contained 3 adults, relationships among them were determined by examining their microsatellite genotypes. Foxes were considered to be neighbors if they lived on adjacent home ranges with a common boundary or partial overlap as determined by the convex polygon method and nonneighbors if they did not.

**Genetic analyses.**—DNA was isolated from kit fox blood samples using standard protocols (Sambrook et al. 1989). We used 11 microsatellites developed for domestic dogs and foxes that amplified in kit foxes (Table 1). Polymerase chain reaction (PCR) reaction components per either 10- or 25-μl reaction were 1× Perkin-Elmer Taq buffer (Foster City, California), 1 unit of Taq polymerase, 2.0 mM MgCl₂ for locus CPH3 and 1.5 mM for all others, 200 μM of each deoxynucleotide, 1 μM of each primer, and 1.7 mg/ml bovine serum albumin (BSA). PCR reactions were cycled 35 times, with denaturation at 94°C for 30 s, annealing at 50–60°C, depending on primer pair, for 60 or 75 s, and extension at 72°C for 2 min. Locus CXX250 was annealed at 50°C; CPH3 and CXX20 at 52°C; CXX173, CXX140, CXX403, CXX263, and FH2054 at 55°C; CXX172 and FH2140 at 58°C; and CXX30 at 60°C. Products were run on agarose minigels in TBE buffer to assess optimal conditions.

Each amplified microsatellite was visualized and checked for polymorphism by 1 or both of 2 methods: Amplification products were run on 8% native polyacrylamide gels in 1× TBE, stained with ethidium bromide, and photographed (only locus CPH3), or fluorescent dye-conjugated nucleotides (dyes TAMRA, RG6, or R110) were incorporated into the PCR reactions, and resulting products were electrophoresed in an 373 Automated Sequencer (Applied Biosystems, Foster City, California). Microsatellite sizes were estimated by comparison to size standards for manual gels and Genescan-500 ROX for automated gels. Automated gel results were analyzed using Genescan 2.1, but most scoring by peak height also was confirmed by examining the gel image. Genotypes were scored for each individual. Genotypes of all 3 pups captured were consistent with the genotypes of their presumed parents, providing limited evidence that the microsatellite alleles we studied were inherited in Mendelian fashion. We used the program GENEPOP (Raymond and Rousset 1995) to ascertain if alleles at each of the microsatellite loci deviated from Hardy–Weinberg proportions and Weir and Cockermah's (1984) estimator to calculate Fₛ for each locus.

Allelic frequencies at each locus were calculated from the entire sample and entered into the program Kinship 1.1.2 (Goodnight and Queller 1999). Kinship 1.1.2 estimated Grafen's relatedness coefficient (r) between 2 individuals, which measured the extent to which they possess alleles that were identical by descent, using allelic frequencies in the population and each in-

<table>
<thead>
<tr>
<th>Locus</th>
<th>n</th>
<th>Alleles</th>
<th>Hₑ</th>
<th>Fₛ</th>
<th>HW</th>
</tr>
</thead>
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<tr>
<td>CPH3ᵇ</td>
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<td>4</td>
<td>0.53</td>
<td>+0.088</td>
<td>0.14</td>
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<td>3</td>
<td>0.27</td>
<td>+0.114</td>
<td>0.08</td>
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<td>4</td>
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<td>0.00</td>
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<tr>
<td>CXX172ᵇ</td>
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<td>2</td>
<td>0.40</td>
<td>+0.075</td>
<td>0.68</td>
</tr>
<tr>
<td>CXX403ᵇ</td>
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<td>4</td>
<td>0.62</td>
<td>+0.179</td>
<td>0.05</td>
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<tr>
<td>CXX263ᵇ</td>
<td>34</td>
<td>2</td>
<td>0.09</td>
<td>−0.031</td>
<td>1.00</td>
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<tr>
<td>CXX30ᵇ</td>
<td>34</td>
<td>3</td>
<td>0.53</td>
<td>−0.065</td>
<td>0.01</td>
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<tr>
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<td>23</td>
<td>4</td>
<td>0.74</td>
<td>+0.475</td>
<td>0.00</td>
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<tr>
<td>CXX250ᵇ</td>
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<td>3</td>
<td>0.32</td>
<td>−0.194</td>
<td>0.63</td>
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<tr>
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<td>6</td>
<td>0.58</td>
<td>−0.262</td>
<td>0.71</td>
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<tr>
<td>FH2140ᵉ</td>
<td>34</td>
<td>7</td>
<td>0.69</td>
<td>+0.019</td>
<td>0.92</td>
</tr>
</tbody>
</table>

| X̄    | 32.3 | 3.8 | 0.50 | +0.097 |
| SE    | 0.5  | 0.06 |

ᵇ Ostrander et al. (1993).
ᵉ Francisco et al. (1996).
individually's multilocus genotype (Queller and Goodnight 1989). Specifically, $r$ was calculated as

$$r = \frac{\sum \sum (P_x - P^*)}{\sum \sum (P_x - P^*)},$$

where $P^*$ was the frequency of each allele in the population (excluding compared individuals) and $P_x$ and $P_y$ were the frequency of each allele in compared individuals (Goodnight and Queller 1999). This measurement of relatedness ranged from $-1$ to $+1$. A positive $r$-value indicated that 2 individuals shared more alleles that were identical by descent than expected by chance, whereas a negative $r$-value indicated that 2 individuals shared fewer such alleles than expected by chance. First-degree relatives such as parents and offspring or full siblings should have an $r$ of 0.5, and pairs of randomly chosen individuals should have an $r$ of 0.

The program Kinship also calculated the likelihood that a pair of genotypes fits a particular hypothesized relationship, either a null ($r = 0$) or alternative ($r = 0.5$) hypothesis. The log of the ratio of those likelihoods indicated whether the null or alternative hypothesis (or neither) was favored. The program performed a simulation based on allelic frequencies entered by the user and hypothesized relationships. We repeated this simulation 5,000 times to provide a distribution of log likelihoods and determined a 0.05 significance level from that distribution. A positive log likelihood larger than that significance level indicated rejection of the null hypothesis and nonrejection of the alternative hypothesis. A negative log likelihood smaller than the negative of the significance level indicated a rejection of the alternative hypothesis and nonrejection of the null. A value between the positive and negative significance levels indicated insufficient power to reject either hypothesis. Thus, a significant $P$-value associated with a positive $r$ indicated rejection of the hypothesis that $r = 0$, and a significant $P$-value associated with a negative $r$ indicated rejection of the hypothesis that $r = 0.5$.

We also used exclusion to test hypothesized parental relationships in the population. An offspring should have had alleles from both of its putative parents: If an offspring had alleles that were not present in 1 or both of its putative parents, then it could be excluded as an offspring of that individual or pair. However, because mutation rates are high in microsatellites, it is possible that a single allele present in neither putative parent represents a mutation rather than nonparentage. Therefore, we only excluded an offspring if ≥2 loci had alleles not present in the putative parent. We assumed parentage when there was a lack of exclusion.

**Results**

*Characteristics of microsatellite loci.*—Expected heterozygosity values ranged from 0.08 to 0.68. Loci with low expected heterozygosity values contributed relatively less to estimates of $r$ produced by the program Kinship. Three loci (CXX30, CXX20, and CXX140) deviated significantly from Hardy–Weinberg proportions (Table 1). Many loci showed a positive $F_{IS}$, with a combined $F_{IS}$ across loci of 0.097, which suggested a deficiency of heterozygotes in the population, and a global test for heterozygosity deficiency was significant ($P = 0.05$).

*Relatedness within social groups.*—To test whether or not mated pairs were closely related, we estimated $r$ for 10 mated pairs. Those values ranged from $-0.52$ to 0.37 (Fig. 1) with a mean of $-0.07 \pm 0.074 SE$. That value did not differ from an $r = 0$ or from the mean of all other pairwise $r$-values ($r = -0.03$, $t = 0.34$, $P = 0.73$, $n = 585$). Distribution of all possible $r$-values excluding mated pairs was centered near 0 and appeared normal (Fig. 1). Thus, mated pairs were not closely related.

To see if extra adults associated with mated pairs were offspring of that pair, we examined 2 social groups containing 3 adults. The 1st trio consisted of an older adult male 108, a younger adult male 103, and the adult female 109. We could not exclude either female 109 or male 108 as a parent of male 103 based on their genotypes; however, we could exclude male 103 as a parent of male 108. Male 108 and female 109 (the mated pair) were not closely related ($r = -0.06$), but both had high coefficients of relatedness with the younger
male 103 ($r = 0.42$ and 0.78, respectively; Table 2). We could reject the null model of $r = 0$ between female 109 and male 103 but not the hypothesis that $r = 0.5$. We could not reject the null model of $r = 0$ between male 108 and male 103. However, that test had little statistical power because alleles that these males shared had relatively high frequencies in the population. Thus, younger adult male 103 was the son of the female 109 and, based on our inability to exclude, also the son of male 108.

The 2nd trio consisted of adult male 133, an older adult female 120, and a younger adult female 121. We could exclude the male as the parent of either female based on genotypes. However, the genotype of female 120 was consistent with the hypothesis that she was the mother of female 121. The coefficient of relatedness between the 2 females was 0.61, and we were able to reject the hypothesis that $r = 0$ but not the hypothesis that $r = 0.5$. Thus, in that case, the additional adult was the daughter of the female in the pair but not of her mate.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Male 108</th>
<th>Male 103</th>
<th>Male 104</th>
<th>Female 104</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 109</td>
<td>-0.06</td>
<td>0.78***</td>
<td>-0.22</td>
<td>0.62**</td>
</tr>
<tr>
<td>Male 108</td>
<td>0.42</td>
<td></td>
<td>0.53*</td>
<td>0.10</td>
</tr>
<tr>
<td>Male 103</td>
<td></td>
<td>0.01</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Male 104</td>
<td></td>
<td></td>
<td>-0.05</td>
<td></td>
</tr>
</tbody>
</table>

* $p = 0.05$, ** $p = 0.01$, *** $p = 0.001$.

**Relatedness of foxes on adjacent home ranges.**—Neighboring foxes (i.e., fox dyads living on adjacent or partially overlapping home ranges) were more closely related (mean $r = 0.12 \pm 0.076 SE$) than nonneighboring foxes ($-0.04 \pm 0.014$; $t = 2.069$, $d.f. = 592$, $P < 0.05$). Neighboring females ($0.37 \pm 0.127$; $n = 5$ dyads) were more related than neighboring male–female dyads ($-0.02 \pm 0.124$; $P = 0.040$, $n = 8$) and nonneighboring dyads ($-0.04 \pm 0.014$; $P = 0.007$, $n = 574$) but not neighboring males ($0.11 \pm 0.111$; $P = 0.187$, $n = 7$) based on ANOVA ($F = 2.85$, $d.f. = 3, 590$, $P = 0.037$) and Bonferroni corrected $t$-tests.

**Den-sharing.**—To see if an adult fox shared dens only with other foxes in the same social group (i.e., its mate and their offspring), we examined 3,797 records of ≥2 foxes found in the same den on the same day. As predicted, foxes sharing a den were members of the same social group in the vast majority of these cases (3,692).

Unexpectedly, however, we also obtained 105 records of foxes that were not members of the same social group sharing the same den on the same day. Many of those records involved various members of 2 neighboring social groups, the previously described trio consisting of males 108 and 103 and female 109, and a neighboring pair consisting of
dency, males and females on adjacent home ranges were usually not closely related.

We found a positive $F_{IS}$ and a significant deficit of heterozygotes, consistent with our finding that related foxes often inhabit adjacent home ranges. We essentially collected genetic samples across several clusters of fox home ranges, each cluster containing related foxes. This can be viewed as sampling across subpopulations at a very fine scale, thus creating a heterozygote deficiency due to the Wahlund effect (Wahlund 1928). The Wahlund effect results from combining populations with different allelic frequencies in a single sample. A deficiency of heterozygotes also exists in several other kit fox populations (M. Schwartz and K. Ralls, in litt.). Williams et al. (2000) found a similar heterozygote deficiency in fishers (Martes pennanti) and speculated that it was due to very fine-scale genetic structure within fisher populations, although they did not have supporting behavioral data.

The vast majority of our observations on den-sharing were consistent with the prediction that an adult fox would use the same den on the same day only with foxes in the same social group, that is, its mate and their juvenile or adult offspring (Koopman et al. 1998). Foxes lived in pairs or occasionally in trios and frequently shared dens with their mates and the additional adult in the group if 1 was present. Contrary to our expectations, however, we also observed occasional instances of den sharing between foxes that were not members of the same social group. Most instances of den sharing between individuals in different social groups lasted only a few days. Many of them appeared to be unsuccessful attempts at pair formation. They usually involved an unpaired male and an unpaired female or females to which he was not closely related. One instance involved an unpaired male and a recently paired male and female, 1 involved a paired male with unpaired females, and 1 involved 2 unpaired males that sequentially shared dens with the same unpaired female. Most other instances of den sharing between individuals in different social groups involved various members of a pair and a trio that lived on adjacent home ranges. Both members of the pair were related to $\geq 1$ member of the trio. Similar but much less extensive observations of den sharing between foxes belonging to different social groups led O'Neal et al. (1987) to speculate that kit foxes might have some kind of expanded social system.

Kit foxes are sometimes considered a "solitary" mammal because individuals tend to forage alone (Waser and Jones 1983). Although it is true that kit foxes in the same social group rarely interact during nighttime activity periods (White et al. 2000), our data indicate that kit foxes can maintain social relationships with their adult offspring or siblings that have dispersed to adjacent territories and found mates. Enduring social relationships between adults and their dispersed offspring also have been observed in crab-eating foxes (Cerdocyon thous), a canid that is larger and somewhat more gregarious than kit foxes, living in social groups of 2 to 5 adults $>1$ year of age (MacDonald and Courtney 1996). Furthermore, Insley (2000) recently found that mother–offspring pairs in the northern fur seal (Callorhinus ursinus) retain the ability to recognize each other's vocalizations for $\geq 4$ years. Thus, ability to recognize adult offspring after they have dispersed from their natal home range may be more common than previously expected, which has important implications for the evolution of mammalian social behavior.

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