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Genetic and spatial structure within a swift fox population

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Summary

1. We incorporated spatial data on swift foxes (*Vulpes velox*) with genetic analysis to assess the influence of relatedness between individuals on their social and spatial ecology. We recorded the space use patterns of 188 radio-collared swift foxes in south-eastern Colorado from January 1997 to December 2000. One hundred and sixty-seven foxes were also genotyped at 11 microsatellite DNA loci and the degree of relatedness between individuals was estimated.

2. We described the genetic structure of the population by examining the relatedness of neighbours and the relationship between the spatial and genetic distance of all individuals. We found that close kin appeared to cluster within the population. Neighbours were significantly more related (mean $R = 0.089 \pm 0.01$) than non-neighbours (mean $R = 0.003 \pm 0.01$; randomization test, $P < 0.0002$). Female clusters were more extensive than male clusters.

3. The degree of genetic relatedness among foxes was useful in explaining why foxes tolerated encroachment of their home ranges by neighbours; the more closely related neighbours were, the more home-range overlap they tolerated (Mantel test, $P = 0.0004$). Foxes did not appear to orientate their home ranges to avoid neighbours and home ranges overlapped by as much as 54.77% ($\bar{x} = 14.13\% \pm 0.41$). Neighbours also occasionally engaged in concurrent den sharing.

4. Relatedness influenced the likelihood that an individual would inherit a newly vacated home range, with a mean relatedness of range inheritors to previous owners of 0.333 ± 0.074 . Thus, the genetic structure of the population and interactions between kin were interrelated to space-use patterns and social ecology of the swift fox.

Key-words: kin clusters, relatedness, social tolerance, space use patterns, swift fox.

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Introduction

Mammals interact with their neighbours and other conspecifics in a variety of ways, ranging from the use of non-exclusive home ranges to defence of exclusive territories (Geffen *et al.* 1999). Home-range defence occurs for a number of reasons, including monopolization of mates and defence of other resources such as food and dens (Davies 1978; Messier & Barrette 1982; Gese 2001). Territorial behaviour is costly, however. Time and energy are spent patrolling the territory, and if encounters with neighbours are aggressive, injury or

even death can ensue. Territoriality should occur only when the benefits outweigh the costs (Davies & Houston 1984). The benefits of territoriality will be reduced in cases where resources are abundant and evenly distributed or intraspecific competition for resources is low.

Inclusive fitness may mediate intraspecific competition for resources when relatives are aggregated spatially and likely to interact cooperatively (Hamilton 1964). If kin are less aggressive towards each other than toward non-kin (Waldman 1988), and if inclusive fitness is increased as a result, cooperative behaviours among kin will evolve (e.g. Garza *et al.* 1997; Hoglund *et al.* 1999). Kinship has been used to explain affiliative and cooperative behaviour within and between social groups in a variety of birds and mammals (e.g. Packer *et al.* 1991; Hatchwell *et al.* 2001; Walls & Kenward 2001; Wimmer, Tautz &

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Kappeler 2002). Furthermore, variation in relatedness is hypothesized to correlate with variation in behavioural interactions (Gompper & Wayne 1996). For example, the benefits of cooperating with kin may also influence where individuals settle, thereby affecting population genetic structure. It has been suggested that the kit fox (*Vulpes macrotis* Merriam), a closely related species to the swift fox (*Vulpes velox* Say), has an 'expanded social structure' in which foxes often interact with foxes from neighbouring social groups (O'Neal, Flinders & Clary 1987). This spatial structure may have been influenced by kin effects as kit fox neighbours are often related (Ralls *et al.* 2001).

Here we describe the spatial and genetic structure of the swift fox. The swift fox is one of the smaller North American fox species, inhabits short and mid-grass prairies of North America, and ranged historically from Canada to northern Texas (Scott-Brown, Herrero & Reynolds 1987). They are highly fossorial, using dens year-round (Egoscue 1979). Little is known of the breeding system or intraspecific interactions of this fox, and the genetic structure of swift fox populations has not been investigated previously. We hypothesized that if swift foxes gain benefits by interacting amicably with kin, this cooperative behaviour would be evident in the degree of overlap between home ranges and in its concurrent use. The more closely related neighbouring swift foxes are, the more their home ranges will overlap and the more individual foxes should tolerate use of overlapping areas. We hypothesized further that if this pattern of cooperation exists, it will be apparent in the genetic structure of the population. Because kin receive benefits from being neighbours, dispersing individuals should settle preferentially near relatives creating kin clusters. Thus, an increased tolerance among neighbouring kin will influence both the spatial ecology and genetic structure of swift fox populations.

Methods

STUDY SITE

The study area (Pinon Canyon Maneuver Site, PCMS) is located in Las Animas County, north-east of Trinidad, Colorado. The foxes sampled inhabited an area of 736 km². The climate is semi-arid, with a mean annual precipitation ranging between 26 and 38 cm. Mean monthly temperatures range from -1 °C in January to 23 °C in July. Elevations range from 1310 to 1740 m. The two main vegetation types are shortgrass prairie and pinyon pine (*Pinus edulis*)-juniper (*Juniperus monosperma*) communities (Shaw *et al.* 1989). The study area was used primarily for cattle ranching prior to 1982, at which time the US Army acquired the PCMS.

GENETIC ANALYSES

Genetic relatedness between individual swift foxes within the population on the PCMS was assessed using 11

microsatellite loci. Blood samples were obtained from foxes caught in box traps and were frozen or stored in lysis buffer (Longmire *et al.* 1991) at a 1 : 5 ratio of blood to buffer. Tissue samples were taken from radio-collared animals found dead and frozen until analysis. Fresh scat samples were collected during trapping allowing for positive correlation between the scat and the fox and then frozen.

DNA was extracted from blood and tissue samples using a blood or tissue Qiagen protocol (Qiagen Inc., Valencia, CA, USA), or a phenol/chloroform protocol (Vardenplas *et al.* 1984). DNA was extracted from scat samples in a room dedicated to processing low-quantity samples using standard protocols of a Qiagen stool kit, using multiple negative controls to test for contamination. Samples were amplified through a polymerase chain reaction (PCR) with microsatellite primers (Saiki, Scharf & Faloona 1985). PCR products were first run on a 1.5% agarose gel to test the quality of DNA extractions, and if necessary (i.e. no band of the appropriate size appearing on the gel) a second extraction of the alternate type was performed. When PCR amplification was faint or absent after the second extraction, DNA extracts were concentrated and purified to remove inhibitors using standard GeneClean protocols (Qbiogene, Inc., Carlsbad, CA, USA).

Microsatellite primers developed for the dog genome and used for the closely related kit fox (Östrand, Sprague & Rine 1993; Fredholm & Wintero 1995; Francisco *et al.* 1996; Ralls *et al.* 2001) were optimized for the swift fox samples (Table 1). The following primers were used successfully: CXX20, CXX30, CXX173, CXX263, CXX403, CXX250, CXX109, CXX2062, CXX377, FH2054 and CPH3. Other primers that were tested but not used were CXX123, CXX225, CXX2001 (rejected due to unsatisfactory amplification) and CXX172, CXX200 and FH2140 (rejected due to an insufficient number of alleles). For blood and tissue samples, the 20 µL PCR reactions were cycled 35 times, with denaturation at 94 °C for 30 s, annealing at 51 or 55 °C, depending on the primer pair (Table 1), for 30 s, and extension at 72 °C for 30 s. Each primer was labelled with a fluorochrome (FAM, HEX or TET). Multiplexes of primers were developed to allow multiple loci to be run simultaneously. CXX20/CPH3, CXX109/CXX30, CXX403/CXX173 and CXX2062/CXX250, were run as multiplexes and CXX263, CXX377 and FH2054 were run as singleplexes. The concentrations of reagents that did not vary between reactions were dNTPs (0.25 mM), bovine serum albumin (BSA) (1.01 mg/mL), regular *Taq* buffer (1×), and regular *Taq* (0.5 U). Reagents that varied were the primer concentrations and MgCl₂ (Table 1). For scat samples, the PCR reactions were cycled 55 times, and 0.2 U of Gold *Taq* DNA polymerase replaced regular *Taq*. Microsatellite genotypes were obtained using an Applied Biosystems 377 sequencer (Applied Biosystems) with a GENESCAN 500-Tamra size standard. The genotypes of the individual foxes were obtained using the software programs GENESCAN™

Table 1. Optimization information, polymorphism information, observed and expected heterozygosities, and reagent concentrations for the microsatellite loci

Locus	<i>n</i>	Annealing temp (°C)	No. of alleles	Size range	H _O	H _E	Primer (uM)	MgCl ₂ (mM)
CPH3	167	55	6	151–161	0.647	0.683	0.1	0.25
CXX403	167	55	4	273–281	0.305	0.305	0.2	0.25
CXX263	166	51	4	114–122	0.482	0.611	0.2	0.19
CXX250	166	51	7	132–140	0.440	0.631	0.3	0.38
FH2054	166	51	6	175–187	0.627	0.650	0.2	0.25
CXX20	167	55	9	129–145	0.707	0.719	0.3	0.25
CXX173	167	55	3	124–128	0.317	0.298	0.2	0.25
CXX109	167	55	3	168–172	0.443	0.661	0.3	0.25
CXX30	122	55	11	141–157	0.787	0.828	0.1	0.25
CXX2062	166	55	6	137–154	0.651	0.683	0.1	0.38
CXX377	164	55	8	173–191	0.561	0.642	0.2	0.25
\bar{x}	162		6.09		0.542	0.610		

version 3.1 and GENOTYPER™ version 2.1 (Applied Biosystems). The blood and tissue samples from 20 individuals were re-amplified and the observed error rate per single locus genotype was estimated by calculating the number of errors/number of PCRs. Due to low quality and concentrations of DNA, scat samples were analysed repeatedly and the confidence of the genotypes was estimated using the software package RELIOTYPE (Miller, Joyce & Waits 2002). RELIOTYPE is a program for assessing the reliability of an observed multilocus genotype and for directing further replication if it is not sufficiently reliable. Genotypes were replicated until a confidence level of obtaining a correct multilocus genotype of 99% was obtained (an average of 4.11 PCRs per locus were used for scat samples). For some samples, unreliable loci were dropped from the analysis. Samples were removed from the analysis unless they were assessed at ≥ 9 loci. Due to possible effects on relatedness values, deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were tested using the program GENEPOP (Raymond & Rousset 1995) and corrected for multiple tests using Bonferroni adjustment.

Relatedness between individuals was assessed using the program KINSHIP™ 1.1.2 (Goodnight & Queller 1999). KINSHIP™ estimates Grafen's relatedness coefficient (Grafen 1985) between all possible pairs of individuals. This coefficient measures the degree to which two individuals share identical alleles, taking into account the allele frequencies in the population and each individual's genotype (Goodnight & Queller 1999). Loci exhibiting lower than expected heterozygosity levels contribute less to the calculation of *R* than loci with higher levels of heterozygosity. *R*-values range between –1 and 1. A positive *R*-value between two individuals indicates that they are more related (i.e. they share more alleles that are identical by descent) than expected by chance, and a negative *R*-value indicates that they are less related than expected by chance. These calculations are based on a reference set which was made up of all sampled foxes. It is important to note that these do not constitute an isolated population, however.

INTERACTION ANALYSES

The interaction of swift fox neighbours was documented using radiotelemetry. Foxes were captured using double-door box traps (80 × 25 × 25 cm) baited with chicken (Covell 1992). Traps were deployed in the evening and checked the following morning. Trapping ceased during periods when night-time temperatures dropped below –10 °C. A radio-collar and ear tag was attached to the fox and the weight, sex and age of the animal was recorded. To recapture certain individuals in order to change their radio-collar, a trap-enclosure system as described by Covell (1992) was used. All foxes were released at the site of capture. The foxes were caught in five areas on the study site. The boundaries of the five areas were > 1.5 home-range diameters apart, and although some foxes dispersed between areas, no fox inhabited more than one area at a time (Schauster, Gese & Kitchen 2002).

Telemetry procedures followed recommendations by White & Garrott (1990). Radio-tagged foxes were monitored to determine home-range estimates, overlap and range inheritance. Relocations on the foxes were attempted approximately every 1–3 days with locations obtained throughout the 24-h period to reduce bias in home-range estimates. Home-range estimates were obtained by a 95% fixed-kernel range estimator (Worton 1989) with least-squares cross-validation smoothing. A social group was defined as foxes that shared a range and concurrently shared dens (Kitchen, Gese & Schauster 1999). Foxes were considered neighbours if they were residents in adjacent ranges with a common boundary or some overlap, as determined by the 95% fixed-kernel home-range estimator.

We compared the relatedness between neighbours to that between non-neighbours. We evaluated the level of overlap between neighbours of different sex combinations (e.g. male–male, female–male and female–female), and whether the relatedness of neighbours correlated with the level of overlap between their ranges. Mean overlap was calculated using an index that varies

between 0 and 100, with 100 indicating complete overlap (Minta 1992).

$$\text{Mean overlap} = \frac{\text{HROverlap} * 100}{\sqrt{\text{HR(A)} * \text{HR(B)}}$$

We assessed whether observed overlap among neighbouring foxes was different from an estimate of overlap expected by chance derived by rotating the ranges randomly about their centroids (Geffen & Macdonald 1992). We compared the actual overlap with the average overlap calculated from randomly rotating one of the ranges three times. If the overlap observed was greater than that expected, individual foxes were considered to be attracted to one another, whereas if the observed overlap was less than expected foxes were assumed to be avoiding one another (Madison 1980). The actual overlap was used instead of the Minta index (Minta 1992) for overlap, as the size of the neighbouring home range was inconsequential to the orientation of a fox's home range.

To evaluate whether foxes temporally avoided their neighbours, we assessed how much neighbouring foxes were using the overlapping areas of their ranges concurrently. To do this, we calculated the simultaneous use of the overlapping area of their range by calculating the percentage of the total points (simultaneous and non-simultaneous) in the overlapping area when both animals were found there within 1 h of each other (deemed simultaneous). This was compared to the percentage of the total points in the non-overlapping area when both animals were located within 1 h using a mixed factor analysis of variance model. The sex combination of the neighbours (female and female, male and female, male and male) was incorporated into the model. Five randomly chosen overlapping neighbours per season per year were used for these analyses. We assessed whether the level of temporal avoidance displayed by neighbours (i.e. the difference between the simultaneous use of overlapping areas vs. non-overlapping areas) was correlated to their relatedness using a Pearson product-moment correlation. We also assessed the frequency of occurrence of den-sharing between neighbours and the relatedness between neighbouring animals that engaged in concurrent den-sharing.

We evaluated the relatedness between swift foxes that inherited empty ranges and the previous owner and compared this to the average population relatedness (the mean relatedness of all dyads of sampled foxes). We assessed this in cases where an apparently single range-holder died, or a mated pair died within a short period. In the latter case, the relatedness of the last pair-member to die was used. If a mated pair began to utilize an empty range at the same time, the more closely related of the members to the former range-holder was used. This method was chosen as swift fox pairs are generally unrelated (Kitchen 2004); thus, only one individual would be related to the previous home-range owner. We believe this method to be valid and did not introduce

bias into the analysis; the average R -value of single inheritors (mean $R = 0.381 \pm 0.093$, $n = 7$) was higher than the more closely related of the members of a mated pair (mean $R = 0.217 \pm 0.108$, $n = 3$).

If kin settled near each other, we hypothesized that distance between home-range centroids would be correlated with genetic relatedness. That is, the more closely related foxes were to one another, the closer their home ranges would be. Distance was grouped into < 3 km (approximately distance between neighbour centroids), 3–6 km (approximate distance between neighbours twice removed) and more than 6 km. Because of the issue of pseudoreplication in the multiple pairwise comparisons of fox relatedness, we used Mantel's randomization tests (Mantel 1967) and analysis of variance and two-sample tests by randomization performed in the software program RT version 2.1 (Manly 1997) instead of conventional statistical tests. These randomization tests were carried out with 5000 permutations to assess: (1) the relatedness of male individuals to other males vs. female individuals to other females within the population; (2) the relatedness of neighbours vs. non-neighbours; (3) the relationship between geographical distance and relatedness of individuals; and (4) whether there was a relationship between home-range overlap and relatedness. Probability values in multiple comparisons were adjusted using a Bonferroni adjustment.

For the purposes of analyses, we defined seasons on the basis of energetic demands (due to climatic changes and prey abundance) and behavioural characteristics (including breeding, gestation, pup-rearing and dispersal) which were applicable to swift foxes as follows: pup-rearing season (15 April–14 August), dispersal season (15 August–14 December) and breeding/gestation season (15 December–14 April). Spatial analyses were performed using ARCVIEW version 3.0 (Environmental Systems Research Institute, Inc., Redlands, CA, USA). Statistical analyses were performed using SAS (2001). Data were examined for normality and homoscedasticity in all parametric tests. Data were log-transformed for assessment of spatial avoidance owing to deviations from normality.

Results

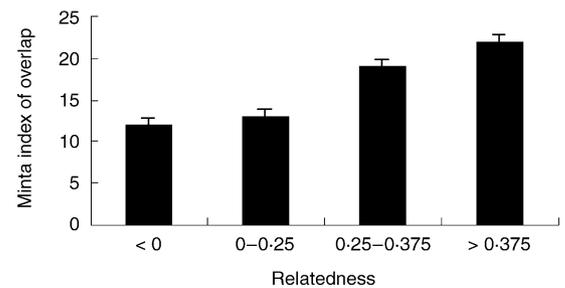
We obtained 32 556 locations on 188 swift foxes, with continuous data collection from January 1997 to December 2000. We analysed genetic samples on 167 foxes. The mean proportion of individuals genotyped at each locus was 0.972, and 164 individuals were genotyped at ≥ 10 loci. The error rate of genotyping was estimated at 0.9%. Forty-four of 52 scat samples met the data reliability criteria discussed in the Methods; the remaining samples were omitted from analysis. Observed heterozygosities per locus varied from 0.305 to 0.787 with an average of 0.542 (Table 1). Significant deviations from Hardy-Weinberg equilibrium were observed at five of the 11 loci when testing the population

Table 2. Exact test *P*-values for Hardy–Weinberg (HW) equilibrium for the whole population and each area individually adjusted for multiple comparisons using Bonferroni adjustment. Values marked * indicate significant deviation from HW equilibrium at the level of 0.05

Locus	Whole population	Area 1	Area 2	Area 3	Area 4	Area 5
CPH3	0.024*	1.000	1.000	1.000	1.000	1.000
CXX403	1.000	1.000	1.000	1.000	0.822	1.000
CXX263	0.000*	0.166	0.333	1.000	1.000	1.000
CXX250	0.000*	0.004*	0.006*	0.670	1.000	0.022*
FH2054	0.782	1.000	1.000	1.000	1.000	1.000
CXX20	1.000	0.854	1.000	1.000	1.000	1.000
CXX173	1.000	1.000	1.000	1.000	1.000	1.000
CXX109	0.000*	0.414	1.000	1.000	1.000	0.054
CXX30	0.074	0.315	1.000	1.000	1.000	1.000
CXX2062	1.000	1.000	1.000	1.000	1.000	1.000
CXX377	0.041*	0.554	1.000	0.482	1.000	1.000

as a whole after Bonferroni adjustment for multiple tests. When the Hardy–Weinberg equilibrium was tested within each of the five sampling areas individually, there was an average of 1.6 loci per area that deviated significantly (Table 2). A number of factors may influence Hardy–Weinberg equilibrium within a population, such as non-random mating. However, because relatives appear to be clustered within our population (see below), the deviations from Hardy–Weinberg equilibrium seen in our population may be due to the Wahlund effect (Wahlund 1928). The Wahlund effect occurs when populations with different allelic frequencies are combined in a single sample. The kin clusters were essentially subpopulations within the population. This is supported further by the fact that deviations were reduced when we tested each area separately, and that we had a low error rate reducing the possibility that deviations were due to null alleles. This effect has also been seen in kit fox populations, where similar kin clustering occurs (Ralls *et al.* 2001). Loci pairs that were in linkage disequilibrium at the 0.05 level in the population overall after adjustment for multiple tests using a Bonferroni adjustment were CXX30 and CXX377, CXX2062, CXX173; FH2054 and CXX20, CXX250; and CXX2062 and CXX377. We tested for linkage disequilibrium within each area, and found that there was an average of 1.8 loci pairs in disequilibrium per area (compared to the six pairs that were in disequilibrium in the population overall). Of the six loci out of equilibrium for the entire data set only one pair (CXX30 and CXX2062) was out in more than one area and this pair was out of equilibrium only in areas 1 and 4. Areas 2 and 5 show no disequilibrium. Thus we feel that the observed linkage disequilibrium in our data set is due to population substructure and sporadic effects rather than physical linkage of these loci in the genome.

Females and males had similar relatedness within the population (mean $R = 0.009 \pm 0.004$ vs. 0.008 ± 0.004 ; randomization test, no. of dyads = 999, $P = 0.362$). Neighbours were significantly more related (mean $R = 0.089 \pm 0.01$) than non-neighbours (mean $R = 0.003 \pm$

**Fig. 1.** Average Minta index for overlap (\pm SE) between neighbouring home ranges for four classes of relatedness between swift foxes in south-eastern Colorado, 1997–2000.

0.01; randomization test, no. of dyads = 990, $P < 0.0002$). There was significantly more home-range overlap with increasing levels of relatedness (Mantel test, no. of dyads = 999, $P = 0.0004$, Fig. 1).

Overall, the mean overlap between neighbouring home ranges was $14.13\% \pm 0.41$. The distribution of overlaps was negatively skewed, however; neighbours overlapped each other's ranges by as much as 54.77%. Male–male neighbours exhibited a slightly smaller overlap ($\bar{x} = 13.64\% \pm 0.789$) than female–female neighbours ($\bar{x} = 14.239\% \pm 0.803$) or female–male neighbours ($\bar{x} = 14.308\% \pm 0.589$). This overlap was not significantly different (randomization test, no. of dyads = 999, $P = 0.95$).

When assessing spatial avoidance, we found that the actual overlap ($\bar{x} = 1.20 \text{ km}^2 \pm 0.14$) was not significantly smaller than the overlap seen from the rotated ranges ($\bar{x} = 1.28 \text{ km}^2 \pm 0.13$; $F_{2,93} = 0.02$, $P = 0.89$); thus, the foxes did not appear to be orientating their ranges in such a way as to reduce overlap with their neighbours.

There was significantly less simultaneous use of the overlapping area ($\bar{x} = 3.49\% \pm 0.89$) than there was of the non-overlapping areas of neighbouring home ranges ($\bar{x} = 21.52\% \pm 1.40$; $F_{1,98} = 102.96$, $P < 0.0001$). There was no difference in temporal avoidance for the different sex combinations ($F_{2,98} = 0.43$, $P = 0.653$) or the different seasons ($F_{2,98} = 0.99$, $P = 0.374$). In

addition, there was a significant positive correlation ($r_{58} = 0.31$, $P = 0.028$) between the relatedness of neighbours and the level of simultaneous use of the portion of their home ranges that overlapped relative to the simultaneous use of non-overlapping areas.

Concurrent den-sharing of non-social group foxes occurred nine times, eight times between neighbours and once between a resident and a transient. In seven of the nine cases, the sharing occurred between members of the same sex. Four cases occurred in the breeding season and five in the dispersal season. Concurrent den-sharers from neighbouring ranges were generally not closely related (mean $R = 0.105 \pm 0.177$).

We found that the 10 range inheritors were, on average, highly related to the foxes from which they inherited the range ($\bar{x} = 0.333 \pm 0.074$). This was significantly higher than the average relatedness of all dyads in the sample ($\bar{x} = 0.009 \pm 0.003$; $t = 4.37$, d.f. = 9, $P = 0.0018$). There appeared to be no sex bias in inheritance, with two males and three females inheriting ranges from males, and two males and three females inheriting ranges from females. There were three range inheritors that were collared prior to the death of the range owner. All three range inheritors came from their natal dens; two from neighbouring ranges and one from two home ranges away.

The geographical distance between foxes' home-range centroids was correlated to relatedness, with higher levels of relatedness among foxes with geographical proximity for all sex combinations (Table 3, Fig. 2). Females exhibited a more extensive kin clustered pattern with higher relatedness extending for larger distances than that seen between males (Table 3, Fig. 2).

Discussion

Our data suggest that swift fox populations are genetically structured at a fine-scale with kin clustering evident. Neighbours were related more closely than expected from the average population relatedness. In addition, foxes were increasingly related as the spatial proximity of their home ranges increased (Table 3, Fig. 2). We propose that the clustering among kin in the swift fox population has led to an increased level of tolerance among neighbours. We found a positive correlation between tolerance levels and the degree of kinship. Overlap of neighbours increased with increasing relatedness (Fig. 1), as did the use of overlapping areas.

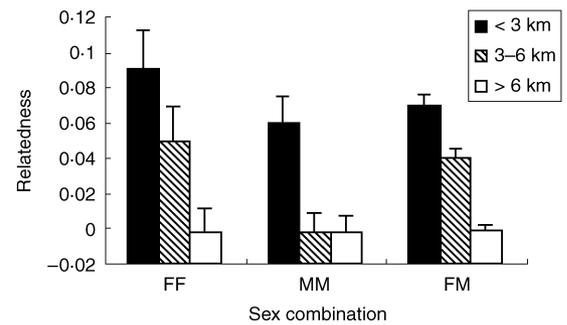


Fig. 2. Average relatedness for three classes of distance between home-range centroids for female–female (FF), male–male (MM) and female–male (FM) sex combinations of swift foxes in south-eastern Colorado, 1997–2000.

While the influence of relatedness on tolerance has not always been clear in field studies (e.g. Spong & Creel 2004), a positive correlation between cooperative behaviour and relatedness has been noted in carnivores, primates and birds (e.g. Morin *et al.* 1994; Hatchwell *et al.* 2001; Widdig *et al.* 2001; Creel & Creel 2002). Girman *et al.* (1997) found that African wild dog (*Lycaon pictus*) neighbours show higher relatedness than expected by chance, and hypothesized that dispersal to neighbouring packs may reduce the frequency and intensity of interpack encounters.

The tolerance between neighbours and its positive correlation with relatedness in swift foxes demonstrate that kin facilitation (Hamilton 1964) may play an important role in the social ecology and space-use patterns of the swift fox. The spatial organization of kin clusters probably facilitated the foxes by reducing the costs inherent in home-range defence, and also benefited foxes in that empty ranges were often inherited by related foxes. Inheritance of ranges by relatives has been seen in birds (e.g. Emlen 1991; Cockburn 1998) and other canids (Moehlman 1989; Schmidt & Mech 1997). Tolerance extended to den-sharing between neighbours. However, the exhibition of den-sharing in swift foxes with unrelated individuals was unexpected but may be due to the presence of a predator as dens appear to be important in predator avoidance for the swift fox.

The difference between swift foxes and other canids that do not exhibit both spatial–genetic structuring and tolerance of neighbours due to relatedness may be due to either intrinsic behavioural factors or extrinsic

Table 3. Randomization test significance values and number of dyads assessed (restricted to 999) when comparing the average relatedness relative to the distance between their home-range centroids for female–female (FF), male–male (MM) and female–male (FM) sex combinations of swift foxes in south-eastern Colorado, 1997–2000. Values are adjusted for multiple comparisons using a Bonferroni adjustment

Distance (km)	FF		MM		FM	
	<i>P</i>	No. of dyads	<i>P</i>	No. of dyads	<i>P</i>	No. of dyads
< 3 vs. 3–6	0.107	402	0.017	278	0.039	962
3–6 vs. 6 +	0.001	999	0.375	999	0.002	999
Overall	< 0.001	999	< 0.001	999	< 0.001	999

human-caused influences. Tolerance of neighbours will be viable only when breeding opportunities are not limited. Many canids that have been studied genetically live in packs (e.g. coyotes, *Canis latrans*, Lehman & Wayne 1991; Williams *et al.* 2003) or confined areas (e.g. island foxes, *Urocyon littoralis*, Roemer *et al.* 2001); thus, the stronger competition for mating opportunities may have led to decreased tolerance of kin. Kin competition would lead to higher dispersal rates and less spatial–genetic structuring such as that seen in coyotes (e.g. Lehman & Wayne 1991) or, where dispersal is not possible, to decreased tolerance of neighbours such as that seen in the island fox (Roemer *et al.* 2001). Alternatively, the lack of spatial–genetic structuring in many canid populations may be due to the high turnover of individuals resulting from high mortality due to factors such as human exploitation (e.g. Williams *et al.* 2003).

The kin-clustering evident in swift foxes indicates that settlement decisions may be influenced by the relatedness of neighbours, and indeed short-range dispersal was seen in the swift fox population (Schauster *et al.* 2002). Dispersal is influenced by kinship in African wild dogs (Girman *et al.* 1997), with dispersal events often coinciding with a change in dominance hierarchy and dispersers often moving to areas close to relatives. Long-range dispersal events were also documented (Schauster *et al.* 2002) and may be an important mechanism of inbreeding avoidance (Gandon 1999). However, the reduced risk and benefits of kin facilitation when living in clusters of relatives seems to have selected against obligatory long-range dispersal in the swift fox. Alternatively, the kin clustering may have been a result of the dispersal patterns displayed by swift foxes.

There was a difference between sexes in the structure of relatedness within the population with female kin clusters more extensive than male kin clusters. Many species exhibit a sex bias in the degree of philopatry to natal sites or social groups (Greenwood 1980). A bias towards female philopatry is most common among social mammals (Eisenberg 1997), and is typical of small-bodied canids (e.g. red foxes, *V. vulpes*, von Schantz 1981; bat-eared foxes, *Otocyon megalotis*, Nel, Mills & Van Aarde 1984; crab-eating foxes, *Cerdocyon thous*, Macdonald & Courtenay 1996). There was a slight bias towards male dispersal within the swift fox population with more males dispersing than females (Karki 2003).

The tolerance shown to neighbouring related foxes indicates that swift foxes can identify related individuals and maintain long-term relationships with them. Continuing social relationships between adults and dispersed offspring have also been documented in crab-eating foxes (Macdonald & Courtenay 1996), and is likely in kit foxes (Ralls *et al.* 2001). The ability to recognize relatives and adjust one's behaviour accordingly has important implications for the evolution of mammalian social systems.

In conclusion, our data suggests that a kin-clustered structure occurs in swift fox society. We found that tolerance of conspecifics was correlated positively with their degree of kinship. Thus, the indirect or kin-selected

benefits accrued from living in kin clusters may be of fundamental importance to the evolution of swift fox breeding systems and space use patterns.

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