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GENETIC SUBDIVISIONS AMONG SMALL CANIDS: MITOCHONDRIAL DNA DIFFERENTIATION OF SWIFT, KIT, AND ARCTIC FOXES

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Abstract.—Gene flow can effectively suppress genetic divergence among widely separated populations in highly mobile species. However, the same may not be true of species that typically disperse over shorter distances. Using mtDNA restriction-site and sequence analyses, we evaluate the extent of divergence among populations of two small relatively sedentary North American canids, the kit and swift foxes (genus *Vulpes*). We determine the significance of genetic differentiation among populations separated by distance and those separated by discrete topographic barriers. Our results show the among-population component of genetic variation in kit and swift foxes is large and similar to that of small rodents with limited dispersal ability. In addition, we found two distinct groupings of genotypes, separated by the Rocky Mountains, corresponding to the traditional division between kit and swift fox populations. Previous workers have characterized these morphologically similar populations either as separate species or subspecies. Our mtDNA data also suggest that kit and swift fox populations hybridize over a limited geographic area. However, the sequence divergence between kit and swift foxes is similar to that between these taxa and the arctic fox (*Alopex lagopus*), a morphologically distinct species commonly placed in a separate genus. This result presents a dilemma for species concepts, and we conclude that kit and swift foxes should be recognized as separate species.

Key words.—Canids, foxes, gene flow, genetic divergence, mtDNA.

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Studies of terrestrial vertebrates often reveal a correspondence between the extent of genetic divergence among populations and the geographic distance and topographic barriers between them (e.g., Avise et al. 1987). However, gene flow among populations of large, highly mobile terrestrial vertebrates may be minimally restricted by distance or the presence of topographic obstacles, and consequently the influence of genetic drift will decrease as a significant factor in among-population divergence. Past studies of large North American canids suggest that gene flow may occur across the continent and suppress genetic differentiation among even widely separated populations (Wayne et al. 1990; Lehman et al. 1991; Lehman and Wayne 1991; Wayne et al. 1992). For example, in the coyote, *Canis latrans,* widely separated populations show no significant mitochondrial DNA (mtDNA) differentiation. This absence of differentiation is probably caused by the large average dispersal by coyotes (50–100 km) and the recent range expansion of the species into areas previously inhabited by the gray wolf, *Canis lupus* (Lehman et al. 1991; Lehman and Wayne 1991). Dispersal ability of canids is determined primarily by physiological tolerances and body size and secondarily by life history (Gittleman 1985, 1989).

We report on mtDNA differentiation in small North American canids, the swift and kit foxes (genus *Vulpes*). These foxes are small (1.5–3 kg) carnivores that live in the arid regions of the western and south-central United States (fig. 1). Their dispersal distance is limited compared with that of larger canids: an average distance of only 11 km with a maximum observed dispersal of 64 km (O'Farrell 1987). Kit and swift foxes subsist primarily on a mix of vertebrate and nonvertebrate prey (Nowak and Paradiso 1983; O'Farrell 1987; Sheldon 1992) and generally live as single mated pairs with the addition of one or more presumed helpers (Moehlman 1986, 1989).

The taxonomy of kit and swift foxes has been the subject of controversy. Two morphologically distinct assemblages of populations have been defined, kit foxes (*Vulpes macrotis*) to the west of the Rocky Mountains and swift foxes (*Vulpes velox*) to the east (Nowak and Paradiso 1983; Thornton and Creel 1975; McGrew 1979; Dragoo et al. 1990) (fig. 1). A hybrid zone has been de-

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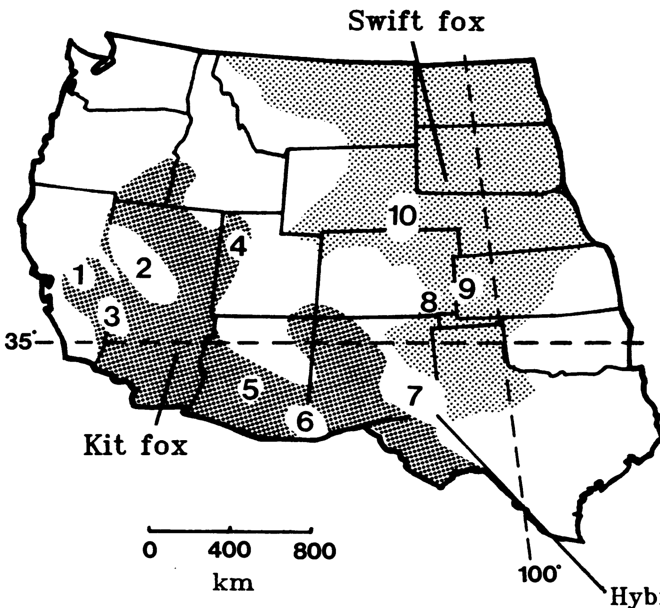
Arctic fox



(1) California, San Joaquin Valley
(2) Nevada
(3) California, Mojave
(4) Utah
(5) Arizona, Central
(6) Arizona, Southeast
(7) New Mexico
(8) Colorado
(9) Kansas
(10) Wyoming

scribed where the ranges of the two forms meet, which has led some authorities to recommend that they be considered a single species, *V. velox* (Packard and Bowers 1970; Rohwer and Kilgore 1973). We will use the term kit-swift foxes when referring to both forms jointly.

Ten subspecies of kit-swift fox have been described (Hall 1981), including the endangered San Joaquin kit fox, *V. macrotis mutica.* However, morphologic and allozyme data suggest most subspecies are not distinguishable, with the exception of the broad groupings of kit and swift fox (Dragoo et al. 1990). Swift foxes first appeared in the mid-Pleistocene about 500 kya and kit foxes first appeared about 100–200 kya (Kurtén and Anderson 1980). Molecular-genetic data suggest the closest living relative of kit-swift foxes is the arctic fox, *Alopex lagopus,* whose circumpolar distribution nearly meets the northern reaches of the historical swift fox range (fig. 1; Wayne and O'Brien 1987; Wayne et al. 1987). Similarly, mtDNA sequence data indicate a very recent divergence of kit-swift and arctic foxes, although morphologically they are very distinct and have been assigned to separate genera (Clutton-Brock et al. 1976; Van Gelder 1978; Geffen et al. 1992).

We test three hypotheses concerning genetic divergence among kit-swift fox populations. First, we assess whether the smaller dispersal abilities of kit-swift foxes are associated with greater among-population differentiation than in larger canids. Second, we determine if, relative to larger canids, topographic barriers more profoundly affect the genetic structure of kit-swift fox populations. We hypothesize that the Rocky Mountains have effectively interrupted gene flow throughout the Pleistocene such that substantial genetic divergence has occurred between kit and swift foxes. A hybrid zone has been reported in southern New Mexico where the Rocky Mountains are lower and present a smaller barrier to dispersal (Rohwer and Kilgore 1973). Other topographic features, such as the Colorado River, which separates the Arizona populations from those in California, Nevada, and Utah, and the Sierra Nevada Mountains of California, which isolate the San Joaquin kit fox population, may act as barriers to dispersal for small canids, although they apparently have not interrupted gene flow among coyote populations (Lehman and Wayne 1991). Finally, we more precisely test a previous hypothesis that the arctic fox is a recent derivative of swift foxes, having evolved from them during the Pleistocene (Geffen et al. 1992).

We examine these hypotheses through analyses of mtDNA restriction-site data and data from about 800 base pairs (bp) of cytochrome *b* sequence. The mtDNA genome of mammals is maternally inherited clonally, and its DNA sequence evolves at a rate 5–10 times faster than that of the average nuclear gene (Brown et al. 1979). Hence, analysis of mtDNA sequences provides high resolution of genetic differences among closely related populations (Brown 1986; Avise et al. 1987; Moritz et al. 1987; Harrison 1989).

Materials and Methods

Samples

Our localities include populations separated by distinct topographic barriers (the Rocky Mountains, the Sierra Nevada Mountains, and the Colorado River), populations separated primarily by geographic distance rather than habitat or topographic barriers (e.g., Nevada and Utah), and populations in the putative hybrid zone between kit and swift foxes in southeastern New Mexico (table 1, fig. 1). A total of 256 kit-swift foxes from 10 localities were sampled along a transect encircling the Rocky Mountains. The arctic fox samples consisted of animals from the Pribilof Islands, Alaska ($N = 3$), a mainland population near Sudbury, Ontario ($N = 9$), and a single zoo animal of unknown origin ($N = 1$). The tissue samples were whole blood, skeletal muscle, or heart. We also obtained 24 samples from a frozen collection of organs previously used in a protein electrophoretic study (Dragoo et al. 1990), but the DNA in these samples was severely degraded, and we typed each sample as a kit or swift fox genotype using a single diagnostic restriction enzyme, *Bam*HI (see below). An organ sample of the red fox, *Vulpes vulpes* (VVU) was used as an outgroup comparison for the restriction-site analysis and a related taxon, the

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FIG. 1. Present geographic range and sampling localities of kit and swift foxes (Ginsberg and Macdonald 1990). Sample localities 1–6 are in kit fox range, locality 7 is in the kit-swift fox hybrid zone, and localities 8–10 are in swift fox geographic range. Arctic fox range in Canada and Alaska is indicated by dark stippling.

TABLE 1. Subspecies, locality, state, county, sample size, and source of kit-swift and arctic fox samples (see fig. 1). Numbers in parentheses indicate degraded samples typed with only one diagnostic restriction enzyme.

Subspecies	Locality	State	County	Sample size	Source
Kit foxes					
1. *Vulpes macrotis mutica*	San Joaquin	California	San Luis Obispo	29	K. Ralls
			Kern	34 (3)	L. Spiegel
			Kern	12	T. O'Farrell
2. *V. m. nevadensis*	Nevada	Nevada	Storley	2	S. Stiver
			Churchill	6	S. Stiver
			Esmeralda	6	S. Stiver
			Lyon	9	S. Stiver
			Mineral	16	S. Stiver
3. *V. m. arsipius*	Mojave	California	Inyo	2	C. Stockwell
			San Bernardino	1	A. Mercure
4. *V. m. nevadensis*	Utah	Utah	Juab	7	E. Geffen
			Millard	2	E. Geffen
5. *V. m. arsipius*	Arizona (central)	Arizona	Maricopa	6 (1)	E. Geffen
			Mojave	(5)	J. Dragoo
6. *V. m. neomexicana*	Arizona (southeast)	Arizona	Cochise	5	J. Phelps
		New Mexico	Hildago	1 (7)	J. Dragoo
7. *V. m. neomexicana*	New Mexico	New Mexico	Lea	2	L. Killgo
			Chavez	19	L. Killgo
			Lincoln	1	L. Killgo
Swift foxes					
8. *Vulpes velox velox*	Colorado	Colorado	Las Animas	60 (8)	D. Miller
9. *V. v. velox*	Kansas	Kansas	Wallace	10	L. Fox
10. *V. v. velox*	Wyoming	Wyoming	Goshen	1	L. Carbyn
			Laramie	25	L. Carbyn
Total Kit-Swift foxes				256 (24)	
Arctic foxes					
Alopex lagopus		Alaska	Pribilof Islands	3	P. White
		Ontario	Sudbury	9	C. Courtin
		Unknown		1	A. Eisenhawer
Total Arctic foxes				13	

corsac fox, *Vulpes corsac* (VCO), was used as the outgroup for the cytochrome *b* analysis (Geffen et al. 1992).

Laboratory Methods

Total genomic DNA was isolated from organ or skeletal muscle samples or from white blood cells separated from whole blood by phenol-chloroform-isoamyl extraction. The DNA was resuspended in a volume of Tris-EDTA (TE) buffer to a final concentration of approximately 1–2 μg/μL. Two to 3 μg of DNA from each fox were digested separately with the following 17 restriction endonucleases: *Bam*HI, *Bgl*II, *Bst*EII, *Bst*UI, *Dra*I, *Eco*RI, *Eco*RV, *Hha*I, *Hin*dIII, *Hin*cII, *Hpa*I, *Mbo*I, *Msp*I, *Rsa*I, *Stu*I, *Xba*I, and *Xmn*I (Gibco, BRL, and New England Biolabs). After digestion, samples were separated by electrophoresis on 1% agarose gels. Digestions using restriction enzymes with four base-pair recognition sites were precipitated by ethanol before electrophoresis. The DNA was transferred to nylon membranes (Nytran; Intermountain Scientific) and radioactively probed with the entire mtDNA genome cloned from a domestic dog. After autoradiography, fragments were sized according to a molecular weight standard. Fragment sizes were summed for each individual to determine if the entire mtDNA genome was represented (approximately 16.8 kb) and to check for deletions, insertions, and heteroplasmy (cf. Densmore et al. 1985; Boursot et al. 1987). Fragment identity was assessed on the basis of comigration of fragments separated on the same gel.

Two restriction enzymes, *Mbo*I and *Bam*HI, were run on all of the samples, even though the pattern recognized by *Mbo*I, a four base-pair recognition site, was included within the six base-pair recognition site of *Bam*HI. Because of this redundancy in recognition sites, data from

*Bam*HI digestions were not used for phylogenetic analysis or to calculate sequence divergence values. However, *Bam*HI restriction analysis effectively discriminated samples of kit and swift foxes (see below) and was used for this purpose on 24 severely degraded samples, which could not be processed with the complete battery of restriction enzymes.

Restriction-Site Analysis

The restriction fragment patterns of each individual for the 17 restriction enzymes were used to define composite mtDNA genotypes (Lansman et al. 1981, 1983; Bermingham and Avise 1986; Wayne et al. 1989). Restriction-site differences were readily estimated from the fragment patterns because all kit, swift, and arctic fox genotypes differed by the inferred loss or gain of only one or two restriction sites for each restriction enzyme. The red fox often differed by more than two restriction sites, and for this species, we assumed that the minimum number of restriction-site changes had occurred (e.g., Lehman et al. 1991). A per-genotype presence-absence matrix of restriction sites was used to calculate maximum parsimony trees relating mtDNA genotypes with the branch-and-bound option in the PC program PAUP (version 3.0 for the Apple Macintosh, by Swofford 1989). A 50% majority rule consensus tree was produced for the set of most parsimonious trees. One thousand bootstrap samplings of the restriction-site data were used to determine the stability of phylogenetic nodes in heuristic maximum parsimony trees (Felsenstein 1985). The trees were rooted using *Vulpes vulpes* as the outgroup. The percentage of nucleotide divergence was calculated using the restriction-site method of Nei and Li (1979) corrected for different numbers of nucleotides in the recognition sites.

Data on kit-swift fox mtDNA genotype frequency and estimated sequence divergence were used to quantify gene flow and genetic partitioning, using a variation on classical *F*-statistics for mtDNA proposed by Nei and Tajima (1983). In this model, maximum-likelihood estimates of within- and among-population nucleotide diversity (N_{st}) are generated by iteration. These calculations were performed on an IBM PC compatible using the Quick-BASIC program HAPLO written by Lynch and Crease (1990).

Cytochrome b Sequence Analysis

Four universal primers were used to amplify a 398 bp and 402 bp region of the mitochondrial cytochrome *b* gene by the polymerase chain reaction (PCR) (398bp: H15149, Kocher et al. 1989; L14724, Meyer and Wilson 1990; 402bp: H15915 and L15513, Irwin et al. 1991). A double-stranded sequence was amplified first and used in a second PCR to generate a single-stranded template by the unbalanced primer method (Gyllensten and Erlich 1988). Each PCR reaction mixture contained approximately 10 ng of genomic DNA and 1 mM dNTP mix in a reaction buffer of 50 mM KCl, 2.5 mM MgCl2, 10 mM Tris HCl (pH 8.8), and 2.5 units of Taq DNA polymerase in a volume of 50 μL. For the double-stranded amplification, 25 pmol of each primer were used, and for the single-stranded amplification an unequal ratio of 25 to 0.25 pmol was used. Forty cycles of amplification were run in a programmable Perkin-Elmer Cetus DNA thermal cycler as follows: denaturation at 94°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 35 s. The double-stranded products were separated in a 2% Nusieve (FMC Corporation, Rockland, Md.) agarose gel in Tris-acetic acid-EDTA (TAE) buffer and stained with ethidium bromide. The appropriate band was cut out of the gel under UV light and resuspended in 10–100 μL of distilled water. One to 5 μL of the double-stranded product was used to produce a single-stranded template. The single-stranded products were concentrated with Centricon 30 microconcentrators (Amicon), and 7 μL of the concentrated single-stranded product was sequenced using the limited primer in the second PCR (Sanger and Coulson 1975) and a Sequenase kit (US Biochemical).

DNA sequence data were analyzed using unweighted maximum parsimony with the branch-and-bound algorithm of PAUP (Swofford 1989). Bootstrap analysis followed the approach for restriction-site data given above. Sequence data was deposited in GenBank (Accession numbers L11725–L11735).

Results

Sequence Divergence and Phylogenetic Relationships of Genotypes

We found 24 mitochondrial DNA genotypes among the 256 kit-swift fox samples and 3 among the 13 arctic fox samples (table 2). Fourteen of the 17 restriction enzymes used showed polymorphisms. All kit-swift and arctic fox genotypes differed by the loss or gain of one or two restriction fragments for the 14 restriction enzymes that showed polymorphisms (table 2). Restriction-

TABLE 2. Restriction-enzyme patterns defining kit-swift and arctic fox genotypes. Only 14 of the 17 restriction enzymes used showed polymorphisms. The first two letters of kit-swift fox genotype designations indicate localities where genotype is most common. X and Y genotype patterns are common in the arctic fox. Bold indicates genotypes belonging to the swift fox clade (fig. 2).

	Restriction enzymes													
Genotype	*Bst*EII	*Bst*UI	*Hinc*II	*Hpa*I	*Mbo*I	*Rsa*I	*Stu*I	*Xba*I	*Xmn*I	*Eco*RV	*Hha*I	*Msp*I	*Bgl*II	*Cla*I
1 SJ4	B	B	A	B	B	C	A	B	A	A	A	A	A	A
2 SJ5	A	B	A	B	B	C	A	B	A	A	A	A	A	A
3 SJ1	A	B	D	B	B	C	A	B	A	A	A	A	A	A
4 CA6	A	B	A	B	A	C	B	A	A	A	A	A	A	A
5 NV9	A	G	A	B	A	C	B	A	A	A	A	A	A	A
6 NV10	A	A	A	B	A	C	A	B	A	A	A	A	A	A
7 NV13	A	A	A	B	A	C	B	B	A	A	A	A	A	A
8 NV14	A	B	A	B	A	C	B	B	A	A	A	A	A	A
9 NV15	A	G	A	B	A	C	A	A	A	A	A	A	A	A
10 AZ26	A	F	A	B	A	C	A	B	A	A	A	A	A	A
11 AZ20	A	B	C	C	A	C	A	B	A	B	A	C	A	A
12 AZ21	A	B	C	C	A	C	A	B	A	A	A	C	A	A
13 NM23	A	B	A	B	A	X	A	B	A	A	A	C	A	A
14 NM24	A	B	A	B	A	C	A	B	A	A	A	C	A	A
15 AZ24	A	B	A	B	A	C	A	B	A	A	A	A	A	A
16 NM25	A	B	A	B	A	D	A	B	A	A	A	C	A	A
17 NM22	**A**	**D**	**A**	**B**	**C**	**A**	**A**	**A**	**B**	**A**	**B**	**B**	**A**	**A**
18 NV16	**A**	**A**	**A**	**B**	**C**	**A**	**A**	**A**	**B**	**A**	**B**	**B**	**A**	**A**
19 CO37	**A**	**C**	**A**	**A**	**C**	**B**	**A**	**A**	**B**	**A**	**D**	**B**	**A**	**A**
20 CO39	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**A**	**A**
21 CO40	**A**	**C**	**B**	**A**	**C**	**B**	**A**	**A**	**B**	**A**	**D**	**B**	**A**	**A**
22 CO50	**A**	**C**	**B**	**B**	**C**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**A**	**A**
23 WY42	**A**	**E**	**A**	**B**	**C**	**A**	**A**	**A**	**B**	**A**	**E**	**B**	**A**	**A**
24 WY43	**A**	**C**	**A**	**B**	**C**	**B**	**A**	**A**	**B**	**A**	**D**	**A**	**A**	**A**
26 ALA1	A	Y	X	B	X	X	A	X	A	A	X	X	X	X
27 ALA2	A	Y	A	B	X	X	A	X	A	A	X	X	X	X
28 ALA3	A	X	A	B	X	X	A	X	A	A	X	B	X	X

fragment changes were additive. For example, for all restriction enzymes with five and six base-pair recognition sites, each new restriction fragment was the sum of two fragments in another genotype, hence a single site change was inferred. The four base-pair recognition enzymes produced four restriction patterns that required two inferred site changes to convert the pattern to that of another genotype. Our survey included 177 restriction sites, which represent 878 nucleotides or approximately 5% of the canid mtDNA genome (Wayne et al. 1990). The sequence divergence among kit-swift fox genotypes ranged from 0.08% to 1.50%. The average sequence divergence was 0.28% ± 0.13% among kit fox genotypes and 0.31% ± 0.15% among swift fox genotypes. Genotypes from foxes separated by the Rocky Mountains had a greater average sequence divergence of 1.11% ± 0.18% (table 3, fig. 1). The restriction enzymes *Bam*HI, *Xmn*I, *Hha*I, *Mbo*I, and *Rsa*I produced fragment patterns diagnostic of swift and kit fox genotypes (table 2).

Unexpectedly, the arctic fox genotypes (ALA) had a small average restriction-site sequence divergence from both kit and swift fox genotypes of only 1.24% ± 0.16%. Thus, the restriction-site data indicate a level of distinction between species placed in two separate genera (*Vulpes* and *Alopex*) similar to that between taxa sometimes believed to be conspecific (kit and swift foxes). The estimated sequence divergences between the outgroup genotype, the red fox (VVU), and the kit-swift and arctic fox genotypes was large, about 7% (table 3), but less than the value of 12.3% previously estimated from analysis of 398 bp of the cytochrome *b* gene (Geffen et al. 1992).

DNA sequence divergences determined by sequencing 800 bp of the cytochrome *b* gene were similar to those estimated by restriction-site analysis (table 3). Cytochrome *b* sequence divergence values were less than 0.3% within kit and swift fox populations but ranged from 0.5% to 1.1% between populations. The average divergence between genotypes from kit and swift populations was 0.8% ± 0.18%. The average se-

TABLE 3. Sequence divergence between selected mtDNA genotypes based on restriction-site data (above diagonal) and based on sequencing of 800 base pairs of the cytochrome *b* gene (below diagonal). Italics indicate divergence values of swift fox genotypes. See the genotype designations in table 2.

	SJ5	AZ26	AZ24	NM25	*NV16*	*CO40*	*WY42*	*WY43*	ALA1	ALA2	VVU/ VCO
SJ5	—	0.15	0.08	0.30	0.92	1.33	1.00	1.16	1.00	1.16	6.91
AZ26	0.30	—	0.08	0.30	0.91	1.32	1.00	1.16	1.15	1.31	7.10
AZ24	0.30	0.00	—	0.23	0.83	1.24	0.92	1.08	1.08	1.24	7.00
NM25	0.40	0.10	0.10	—	0.76	1.17	0.85	1.00	1.00	1.16	6.68
NV16	0.80	0.50	0.50	0.70	—	*0.37*	*0.22*	*0.37*	0.98	0.98	6.78
CO40	1.10	0.90	0.90	0.80	*0.30*	—	*0.61*	*0.30*	1.39	1.39	7.23
WY42	1.00	0.70	0.70	0.80	*0.10*	*0.40*	—	*0.45*	1.07	1.07	6.98
WY43	1.00	0.70	0.70	1.00	*0.10*	*0.10*	*0.30*	—	1.39	1.39	7.21
ALA1	2.00	1.60	1.60	1.80	1.80	2.10	1.90	1.90	—	0.15	6.72
ALA2	2.00	1.60	1.60	1.80	1.60	2.00	1.80	1.80	0.40	—	6.50
VVU/VCO	12.60	12.50	12.50	12.60	12.50	12.90	12.70	12.60	12.10	12.00	—

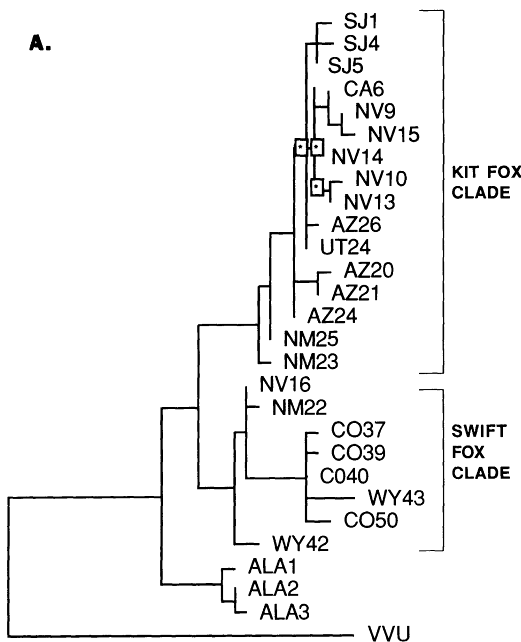
quence divergence between kit-swift fox genotypes and those from the arctic fox was 1.8% ± 0.17%, with a range of 1.6% to 2.1%. The sequence divergence between kit-swift, and arctic fox genotypes and the corsac fox genotype was approximately 12.5%, similar to the 12.0% value previously estimated from an overlapping region of only 398 bp of the cytochrome *b* gene (Geffen et al. 1992). Thus, the DNA sequence data confirm the separate grouping of kit and swift fox populations as well as the close association of both populations to the arctic fox.

Phylogenetic trees based on character-state analysis of the restriction-site and sequence data reflected the relationships suggested by the sequence divergence values (figs. 2, 3). Two clades were apparent in restriction-site and mtDNA sequence trees, corresponding to the populations separated by the Rocky Mountains and designated as kit and swift foxes. The one exception was the genotype 16 (NV16), which was classified in the swift fox clade but was also found in individuals from both sides of the Rocky Mountains (table 4, fig. 2A). Within kit foxes, an additional clade was defined in the restriction-site tree by all three genotypes found in a sample of 75 San Joaquin foxes (fig. 2A). No other clades coincided with the presence of substantial geographic barriers. Finally, arctic fox genotypes (ALA1–3) appeared to define a separate clade that was basal to the clade containing kit and swift fox genotypes (figs. 2, 3).

Bootstrap analysis of restriction-site data for a reduced sample of genotypes indicated that nodes defining kit and swift foxes are found in 95% and 97% of the 1000 heuristic maximum-parsimony trees (fig. 2B). Similarly, a bootstrap analysis of cytochrome *b* sequence data for the eight kit and swift fox genotypes showed that both the kit and swift fox clades are upheld in 81% of the trees (fig. 3). Thus, the nodes defining the kit and swift fox clades are strongly supported by both the restriction-site and cytochrome *b* sequence data (Felsenstein 1985; Sanderson 1989).

Distribution of Genotypes among Localities

Over the combined range of kit and swift foxes, the number of genotypes varied among localities from one in Kansas to nine in Nevada, with an average number of four per locality (table 4). Some genotypes occurred at several localities including NV16 and AZ21, but most were specific to one or two neighboring localities (table 4). Locality-specific genotypes included all three San Joaquin genotypes, one of the Arizona genotypes, and many of those found in Nevada, the California Mojave desert, New Mexico, Colorado, and Wyoming localities. This high degree of geographic partitioning suggests significant population differentiation, estimated here as N_{st}, using the Nei and Tajima (1983) maximum-likelihood approach that considers both the distribution of genotypes among localities and their sequence divergence. The value of N_{st} for our set of localities was 0.85, which is significantly different from zero ($P = 0.01$). Similarly, when kit and swift fox ranges were analyzed separately, the N_{st} values were 0.75 and 0.65, respectively, both highly significant ($P = 0.01$). Thus, genetic variation in both kit and swift foxes was highly structured with more than 65% of the variation being explained by the among-population component.



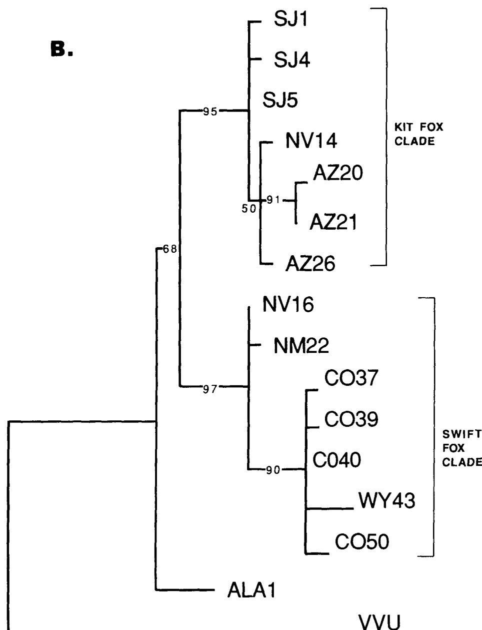


FIG. 2. (A) A 50% majority-rule consensus tree of 30 most parsimonious trees of kit and swift fox genotypes based on analysis of restriction site data. Nodes indicated by stars are supported only in 60% of trees, other nodes are found in all 30 most parsimonious trees. The tree was generated using the branch-and-bound option of the PAUP program (Swofford 1989) and rooted by the red fox (VVU) restriction site data. For genotype codes see table 2. Tree length = 91, overall consistency index (CI) = 0.82, CI excluding uniformative characters = 0.66, rescaled consistency index = 0.74.

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(B) Bootstrap 50% majority-rule consensus tree of select kit-swift and arctic fox genotypes. The percentage of 1000 heuristic bootstrap trees in which a node is supported is indicated. Tree length = 84, overall consistency index (CI) = 0.86, CI excluding uniformative characters = 0.65, rescaled consistency index = 0.75.

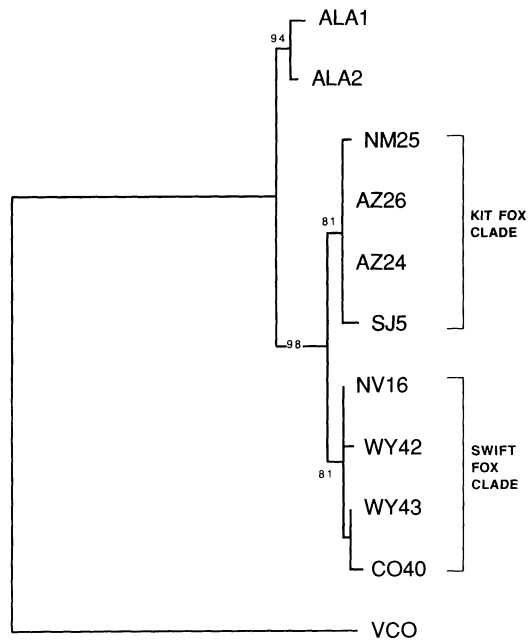

FIG. 3. The single most parsimonious tree based on phylogenetic analysis of 800 base pairs of the cytochrome *b* gene of selected kit, swift, and arctic fox genotypes defined by restriction site data. Percentages of 1000 heuristic bootstrap trees in which a node is supported is given below each node. Outgroup for sequence analysis is *Vulpes corsac* (VCO). For genotype codes see table 2. Tree length = 103, overall consistency index (CI) = 0.98, CI excluding uniformative characters = 0.88, rescaled consistency index = 0.92.

We also examined geographic partitoning by subsampling the locality data and plotting the average number of genotypes added to the survey for each additional locality (Lehman and Wayne 1991). The curves relating these variables for deer mice (*Peromyscus maniculatus,* Lansman et al. 1983), coyotes (*Canis latrans,* Lehman and Wayne 1991), and the kit-swift foxes showed that for the scale of sampling used in this study, the rate of addition of genotypes per-locality in kit-swift foxes was as great as that of deer mice (fig. 4). The high level of genetic structuring in kit-swift foxes reflects the presence of multiple genotypes at each locality that are not widely shared

TABLE 4. Genotype abundance at sampling localities (fig. 1). Starred genotypes are classified in the swift fox clade (fig. 2).

Genotypes	San Joaquin	Mojave	Nevada	Utah	Arizona (central)	Arizona (SE)	New Mexico	Colorado	Kansas	Wyoming	Total
SJ1	2										2
SJ4	20										20
SJ5	53										53
CA6		3									3
NV9			1								1
NV10			12								12
NV13			1								1
NV14			1								1
NV15			2								2
NV16*			5				8	31	10	15	69
UT24			11	8							19
AZ21			3	1	4	2					10
AZ20						3					3
AZ24			3			1					4
AZ26					2						2
NM22*							1				1
NM23							2				2
NM25							11				11
CO37*								4			4
CO39*								2			2
CO40*								22			22
CO50*								1			1
WY42*										5	5
WY43*										6	6
Total	75	3	39	9	6	6	22	60	10	26	256

among localities. In deer mice, only one unique genotype was generally found at each locality (52 of 61 localities had unique genotypes, Lansman et al. 1983). Using the equation describing the kit-swift fox curve, we estimate the total number of genotypes in kit-swift foxes is 93, more than in any other canid species studied to date (Wayne et al. 1989; Lehman et al. 1991; Lehman and Wayne 1991; Wayne et al. 1992).

Sequence divergence among genotypes from different localities often increases with geographic distance and the presence of topographic barriers between localities. This correspondence of sequence divergence and geographic distance or topography has been labeled phylogeographic partitioning (Avise et al. 1987). Because several genotypes sometimes occurred at a single locality, and some were shared among localities, we examined phylogeographic patterns in kit-swift foxes by computing the average nucleotide similarity between the sampling localities and generating a tree using UPGMA cluster analysis (Lynch and Crease 1990; Lehman and Wayne 1991). This UPGMA tree, based on average nucleotide similarity, again indicated groupings of eastern (Wyoming, Kansas, Colorado, and New Mexico) and western (Utah, California, Nevada, and Arizona) populations corresponding to kit and swift fox geographic ranges (figs. 1, 5). Within the kit fox cluster, the Arizona population formed a distinct cluster that was separate from a cluster containing all of the populations on the opposite side of the Colorado River. The New Mexico locality, which is located in the hybrid zone between kit and swift foxes, appears as the most distinct locality in the swift fox group and contains genotypes assigned to both kit and swift fox clades (fig. 2A, table 4).

Finally, the number of mtDNA genotypes within the kit and swift fox clades differed. Kit foxes had twice as many genotypes (16) as the swift foxes, although within-clade divergences were similar (less than 0.5%; table 3). This result could reflect differences in sample size and the number and distribution of sampling localities. Excluding the samples from New Mexico where the two forms meet, we analyzed 138 samples from six distinct localities within the kit fox geographic range and 96 samples from three localities within the swift fox geographic range. The relationship between the number of genotypes added for each additional locality (fig. 4), sug-

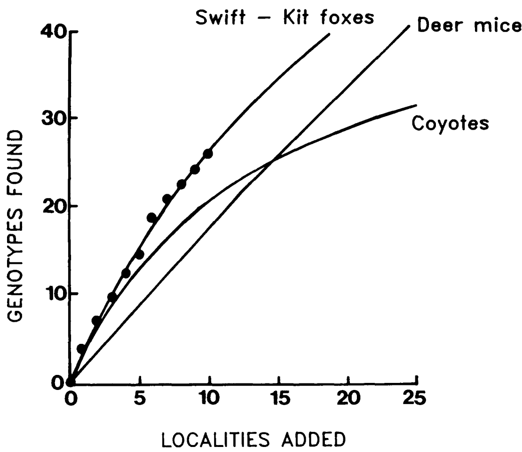

FIG. 4. The increase in the total number of genotypes added per locality. This relationship was determined by a Monte Carlo simulation involving the selection of kit-swift fox localities at random without replacement and summing the number of genotypes found with each added locality (Lehman and Wayne 1991). Circles represent the average for 10,000 replicate runs of the simulation and the line fitted through the points is a curve with equation $y = ax/(x + b)$ where a is the asymptote, which here equals 93 genotypes.

gests that adding three localities to our swift fox sample might make the number of genotypes found nearly equivalent in kit and swift foxes.

DISCUSSION

Body Size, Dispersal Distance, and Gene Flow

Canids are a morphologically uniform group, and in general, limb and cranial proportions follow strict allometric trends (Wayne 1986a,b). Body-size related trends are also evident in prey size, home range area, and dispersal distance (Moehlman 1986, 1989; Gittleman 1985, 1989). Although few quantitative data exist, the physical constraints imposed by body size on dispersal distance and on the ability to surmount physical obstacles are apparent. Larger body size may provide more endurance and better buffering capability against environmental variables such as temperature, food, and water stress (Gittleman 1985). Other factors that also affect dispersal may be size independent. For example, the habitat specificity of kit-swift foxes may be an important factor restricting their dispersal relative to slightly larger foxlike canids that are habitat generalists (see Sheldon 1992).

Because average dispersal distance in terrestrial mammals tends to decrease as body size decreases, the magnitude of the component of genetic variation reflecting population subdivision, F_{st}, should show a corresponding increase. F_{st} can be estimated in a continuum model as $1/[1 + 4c(\pi D\sigma^2)]$, where c is a constant, D is a measure of the density of demes, and σ^2 is dispersal distance variance (Slatkin and Barton 1989). In small canids, smaller dispersal distances should lead to larger values of F_{st}, although a corresponding increase in the density of demes could reduce this effect. Our values of N_{st} in the kit-swift fox (≈ 0.85), higher than in large canids sampled over a similar geographic scale (coyote ≈ 0.29, Lehman and Wayne 1991), suggest that the smaller dispersal distances of kit-swift foxes are not accompanied by a corresponding increase in deme density. F_{st} may also be related, in a stepping-stone model, to the number of individuals that move among demes as $1/(1 + 4Nm)$, where N equals effective population size and m equals migration rate (Slatkin 1987). The estimate of F_{st} provided by N_{st} in coyotes is about 0.29, and thus $Nm = 0.61$ (Lehman and Wayne 1991). In the kit-swift fox, N_{st} is approximately 0.85, and thus $Nm = 0.044$. Values of Nm much less than 1, as in the kit-swift fox, imply that genetic drift will be the principal force structuring among-population variation at neutral loci (Slatkin 1987).

Influence of Topographic Barriers on Genetic Differentiation and Hybridization

The primary barrier obstructing gene flow in kit-swift foxes is the Rocky Mountains. Isolation of populations on both sides of this barrier has led to differentiation as evidenced by mtDNA restriction-site sequence divergence and sequence divergence of 1% in about 800 bp of the cytochrome *b* gene. Differentiation is apparent in morphology as well. Older morphologic studies showed that kit foxes are smaller than swift foxes and have more pointed muzzles, larger ears, and longer tails (Thornton and Creel 1975; McGrew 1979). However, recent analysis of cranial measurements and allozyme data suggested that only small differences exist between the two forms (Dragoo et al. 1990). Our results support the older studies, as kit and swift fox genotypes formed distinct well-supported clades in a phylogenetic analysis of both restriction-site and cytochrome *b* sequence data. Furthermore, the degree of divergence between the kit and swift fox

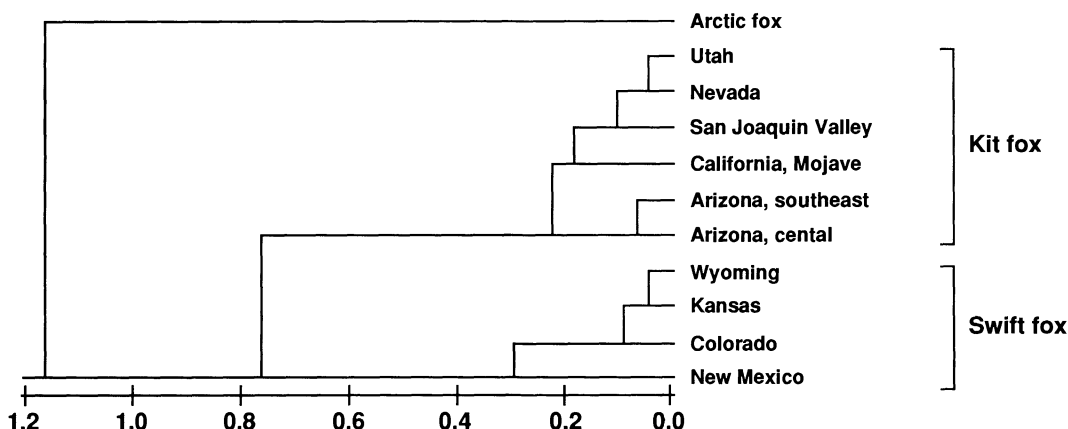

FIG. 5. UPGMA tree based on the average between-locality nucleotide similarity values for the 10 kit-swift fox localities. Average nucleotide similarities are also computed relative to the arctic fox genotype ALA 1 (table 2). Scale is least squares fitted sequence divergence values (Lynch and Crease 1990).

clades was similar to that between kit-swift foxes and the arctic fox, a species often placed in a separate genus.

A second, less significant barrier to gene flow appears to be the mountains to the south and east of the San Joaquin Valley in California. The Tehachapi Mountains bounding the southern end of the valley are lower than the Sierra Nevada Mountains to the east and may form a more permeable barrier. Although past morphologic studies suggested that the San Joaquin kit fox is a morphologically distinct taxon (Waithman and Roest 1977; Hall 1981), recent morphologic and allozyme analyses did not distinguish it from populations outside the San Joaquin Valley (Dragoo et al. 1990). We analyzed a large sample of 75 San Joaquin kit foxes from the southern part of this taxon's range and found three distinct genotypes that formed a consistent clade (fig. 2A). This clade is supported only by two restriction-site changes (table 2) and is not significant in a bootstrap analysis (fig. 2B). However, relative to variation within kit foxes, the San Joaquin clade appears as the most distinct single phylogeographic unit and is an isolated population, indicating that it should be considered a subspecies (Avise and Ball 1990). None of the other 10 subspecific designations of kit and swift fox (Hall 1981) are readily supported by our data.

The only other important barrier to dispersal and gene flow may be the Colorado River. The two kit fox populations from Arizona, on the eastern side of the river, share genotypes with the Nevada population on the west side but also have genotypes not found elsewhere (table 4). The sequence similarity of genotypes in the two Arizona populations causes them to be clustered together in a UPGMA analysis of average sequence similarity among localities. More extensive sampling is needed to define better patterns of microgeographic variation caused by barriers such as the Colorado River.

The mtDNA analysis confirms that hybridization between kit and swift foxes may occur in southeastern New Mexico where the ranges of the two forms meet (fig. 1). Forty-one percent of the foxes there ($N = 22$) had genotypes from the swift fox clade, and 59% had genotypes from the kit fox clade (table 4). Populations in southeastern Colorado and western Kansas, approximately 400 km to the north, contained only swift fox genotypes ($N = 78$), and the southeastern Arizona sample, approximately 440 km to the west, contained 92% kit fox genotypes ($N = 13$) (fig. 6). These observations support a hybrid zone a few hundred kilometers in width and are consistent with descriptions by Packard and Bowers (1970) and Thornton and Creel (1975) of a contact zone between the kit and swift foxes in west Texas and eastern New Mexico.

Previous mtDNA restriction-site analyses have defined two hybrid zones between species of large wolflike canids (Lehman et al. 1991; Wayne and Jenks 1991; Wayne and Lehman 1992). The first hybrid zone includes an area of northern Minnesota and eastern Canada several thousand kilometers in length, where coyotes have recently invaded habitats previously occupied by the gray wolf, *Canis lupus* (Lehman et al. 1991). The second hybrid zone is older and more extensive and

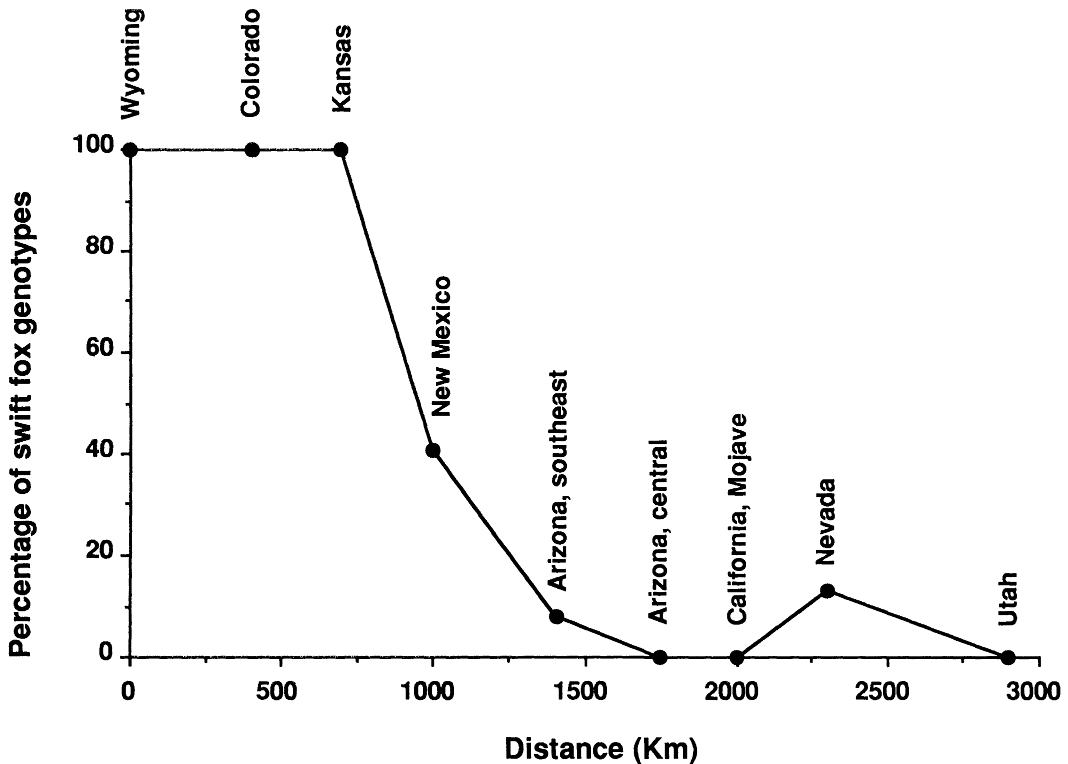

FIG. 6. Percentage of genotypes at each locality belonging to the swift fox genotype clade (fig. 2A) as a function of distance from the Wyoming locality (fig. 1). The 12% of swift fox genotypes found in Nevada is accounted for by the presence of the NV16 genotype.

involves hybridization between coyotes, red wolves (*Canis rufus*), and gray wolves in the American South (Wayne and Jenks 1991). The greater extent of hybridization in large wolflike canids than in the smaller kit-swift foxes may reflect the greater dispersal abilities of the former (Wayne and Jenks 1991) and suggests that interspecific gene flow may constrain speciation in large canids.

The only exception to the absolute partitioning of kit and swift fox clades was the presence of mtDNA genotype NV16 in western Nevada in the kit fox range. This genotype was phylogenetically assigned to the swift fox clade (fig. 2A, table 3). It was the most common swift fox genotype and was found at every swift fox locality sampled, including the hybrid zone in New Mexico. The simplest explanation of this pattern would be the dispersal of swift foxes into the range of the kit fox, followed by hybridization. The shortest route would be directly west across the Rocky Mountains from southwestern Wyoming. Western Wyoming contains marginal swift fox habitat and populations that are isolated and disjunct (Floyd and Stromberg 1981; Linberg 1986). High plateaus in the western part of the state descend into the Great Basin and might provide a corridor for limited gene flow between Wyoming swift fox and western Nevada kit fox populations. Alternatively, a more circuitous southern route for dispersing swift foxes, from New Mexico to the northwest through Arizona, is possible. This route seems less likely because of the absence of NV16 genotypes in the intervening areas and the fact that a dispersing swift fox would also have to cross the secondary barrier posed by the Colorado River. Finally, human-assisted transport cannot be eliminated as a possible mechanism for gene flow between the two forms.

Alternatively, the presence of swift fox genotype NV16 in western Nevada might reflect incomplete lineage sorting following isolation of kit and swift fox populations (Neigel and Avise 1986; Pamilo and Nei 1987; Avise et al. 1990). Initially, isolated populations are likely to pass

through stages of polyphyly, followed by paraphyly, and finally reciprocal monophyly, in which all genotypes in each taxon descend from a separate common ancestor (Neigel and Avise 1986; Avise et al. 1990). The time, in generations, required to reach reciprocal monophyly in a random-mating population is approximately four times the effective female population size (Neigel and Avise 1986; Avise et al. 1988). Assuming an approximate sequence divergence between kit and swift fox genotypes of 1%, and a conversion of 2% sequence divergence per million years of separation (Shields and Wilson 1987), the two populations have been isolated for approximately 500,000 yr. Given a generation time of 1 yr (Ginsberg and Macdonald 1990), we might expect reciprocal monophyly in kit and swift fox populations if the effective population size was less than ¼ × 500,000 or about 125,000 females. The census population size of kit and swift fox populations is not well known, but census population sizes of several hundred thousand adult females are likely (McGrew 1979; Stromberg and Boyce 1986; Scott-Brown et al. 1987). Assuming that the effective population size of females is approximately half of the census adult female population size (Lehman and Wayne 1991), these calculations suggest the possibility that swift and kit fox populations have not attained reciprocal monophyly. Therefore, incomplete lineage sorting remains a possible explanation for the presence of genotype NV16 in the Nevada population (see discussion in Avise et al. 1990).

Evolution of the Kit, Swift, and Arctic Fox and Classification Problems

The first ancestor of kit-swift foxes dates to about 2 mya and is represented by three isolated molars from Blancan age deposits in Scurry County, Texas (Dalquest 1978). The modern swift fox appears about 500 kya in the mid-Irvingtonian from localities in Kansas and more recent deposits in Nebraska. Fossils in the kit fox geographic range are recorded from Rancholabrean deposits about 100–200 kya (Kurtén and Anderson 1980). Arctic fox fossils are first described in the late Pleistocene of Europe about 200 kya and from more recent Wisconsin age deposits in the Yukon (Kurtén and Anderson 1980). The sequence in the ages of arctic fox fossils led to the inference that the arctic fox originated in Europe and is an immigrant to North America (Kurtén and Anderson 1980). However, the mtDNA data suggest that the arctic fox is closely related to the kit-swift foxes, and as the latter have a more ancient fossil record, we have suggested a North American origin for arctic foxes is more consistent with the molecular and fossil evidence (Geffen et al. 1992).

Classification of the kit, swift, and arctic foxes is problematic. Most studies, based on morphological data, have concluded that the arctic fox warrants generic distinction (Huxley 1880; Mivart 1890; Simpson 1945; Clutton-Brock et al. 1976; Van Gelder 1978) and phenotypically, kit-swift and arctic foxes are dramatically different. However, assigning the arctic fox generic status conflicts with the evidence from karyology, DNA hybridization, and allozyme electrophoresis, all of which indicate a close relationship of the three taxa (Wayne and O'Brien 1987; Wayne et al. 1987; Wayne et al. 1989). Our phylogenetic analysis of mtDNA sequence data supports these previous genetic studies, showing that the arctic fox, in spite of its morphological differences, is closely related to the kit and swift foxes. Thus, we conclude that the kit, swift, and arctic foxes should be given similar taxonomic rank because they comprise three closely related clades.

However, should kit and swift foxes be regarded as subspecies of the same species or as distinct species? Widely cited characteristics of a species include the presence of reproductive isolation or species recognition mechanisms (Mayr and Ashlock 1991; Paterson 1985), autapomorphies indicating a monophyletic origin (Cracraft 1983; McKitrick and Zink 1988), phylogeographic concordance (Avise and Ball 1990), evolutionary independence (Simpson 1961), and phenotypic distinction (Sokal 1986). Subspecies have recently been regarded as allopatric and phylogenetically distinct populations that may merge genetically when ecological or topographic barriers are removed (Avise and Ball 1990; O'Brien and Mayr 1991). Kit and swift fox populations comprise distinct clades and are unlikely to merge genetically as they are largely separated by a long-standing barrier, the Rocky Mountains. Even though a narrow hybrid zone exists between kit and swift foxes, gene flow has not obscured the phylogenetic distinction of the two taxa. In fact, hybridization among closely related species is common in vertebrates and the width of the hybrid zone, given equilibrium conditions and the limited dispersal abilities of kit and swift foxes, may not increase over time (Barton and Hewitt 1985, 1989). We suggest that levels of phylogenetic distinction between kit and swift

foxes are likely to increase with time and that the two taxa are unlikely to merge genetically as a result of shifting topographic barriers. Thus, they should be considered separate species in expectation of continued evolutionary independence.

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