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Phylogenetic relationships of the fox-like canids: mitochondrial DNA restriction fragment, site and cytochrome *b* sequence analyses

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(With 3 figures in the text)

The fox-like canids include taxa from the genera *Alopex*, *Otocyon*, *Fennecus*, *Urocyon* and *Vulpes*. Previous morphological analysis indicated that species from the latter three genera are very similar and should be included in the same genus whereas *Alopex* and *Otocyon* are sufficiently different to be included in separate genera. Using phylogenetic methods, we analyse mitochondrial DNA (mtDNA) restriction fragment and restriction site data, and 402 bp of cytochrome *b* sequence variation in fox-like canids. Our results suggest that *Alopex lagopus*, the arctic fox, is actually a very close relative of the swift fox, a species in the genus *Vulpes*. Similarly, the fennec, *Fennecus zerda* is related to the co-existing desert species, the Blanford's fox, *Vulpes cana*. The grey fox, *Urocyon cinereoargenteus*, and the bat-eared fox, *Otocyon megalotis*, are not closely related to each other or to any of the sampled fox taxa. Our results indicate that desert adaptations have evolved independently at least twice in the Canidae, and that Pleistocene glaciations and character divergence may be important causes of morphological change in canids.

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Introduction

The fox-like canids are morphologically very similar carnivores which has led to taxonomic debate about the generic status and evolutionary relationships of several species. The most frequently recognized taxonomic divisions within the group are as follows: *Vulpes*, which includes the red fox, swift fox and seven Old World species; *Urocyon*, the North American grey fox; *Alopex*, the arctic fox; and *Fennecus*, the diminutive desert-adapted fox of the Sahara. The bat-eared fox, *Otocyon megalotis*, is fox-like in some characteristics but is generally thought to represent a separate genus if not subfamily (Simpson, 1945). However, these generic divisions have been challenged by several authors. For example, based on phenetic analysis, Clutton-Brock *et al.* (1976) place *Urocyon* and *Fennecus* in *Vulpes*, leaving intact only *Alopex*. van Gelder (1978) places all the fox-like canids, except *Otocyon* in *Canis*, and places *Urocyon*, *Fennecus* and *Vulpes* in one subgenus separate of *Alopex*. van Gelder uses as a generic criterion the ability to hybridize in captivity or the wild. Recent molecular similarity data (Wayne & O'Brien, 1987) have confirmed the older arrangements of genera (e.g. Simpson, 1945), with the exception that *Alopex* appeared remarkably similar to the swift fox, *Vulpes velox*. Clearly, a rigorous phylogenetic revision of the group is needed so that each genus can be defined as an inclusive monophyletic group.

In this study, we analysed the mitochondrial DNA of 10 species of fox-like canids by restriction site analysis and DNA sequencing of 402 bp of the cytochrome *b* gene. We analyse the restriction site and sequence data using maximum parsimony and maximum-likelihood approaches. Our results demonstrate the phylogenetic distinctiveness of *Urocyon* and *Otocyon* but suggest that the generic distinctions within the remaining fox-like canids are invalid.

Methods

Blood or tissue samples of 10 fox-like canids (Table I, Fig. 1) and a grey wolf were available. For each species we analysed samples from 2–4 different individuals for the restriction fragment and site analysis and one individual for the cytochrome *b* sequence. We considered the swift/kit foxes as one species (*Vulpes velox*; Dragoo *et al.*, 1990) and incorporated into the analysis DNA samples from the most distant ends of its distribution (Colorado and Nevada; Fig. 1). We had only a limited amount of DNA from the bat-eared fox which was used only for the cytochrome *b* sequencing.

TABLE I

Weight, geographic range and number of chromosomes of the fox-like canids that were examined in this study

Scientific name	Common name	Weight range (kg)	Geographic range	Number of chromosomes[a]
Otocyon megalotis	Bat-eared fox	3·0–4·5	East and South Africa	72
Urocyon cinereoargenteus	Grey fox	2·5–7·0	North and Central America	66
Fennecus zerda	Fennec fox	1·0–1·5	North Africa and the Arabian peninsula	64
Alopex lagopus	Arctic fox	3·1–3·8	The Arctic Circle	50
Vulpes velox	Kit/swift fox	1·8–3·0	Western North America	50
V. ruppelli	Ruppell's sand fox	1·1–2·1	North Africa and the Arabian peninsula	40
V. vulpes	Red fox	1·8–5·4	Holarctic	34–38
V. cana	Blanford's fox	0·9–1·4	South-western Asia	?
V. chama	Cape fox	3·6–4·5	Southern Africa	?
V. corsac	Corsac fox	2·7–3·0	Central Asia	?

[a] Chiarelli, 1975; Wayne *et al.*, 1987*b*

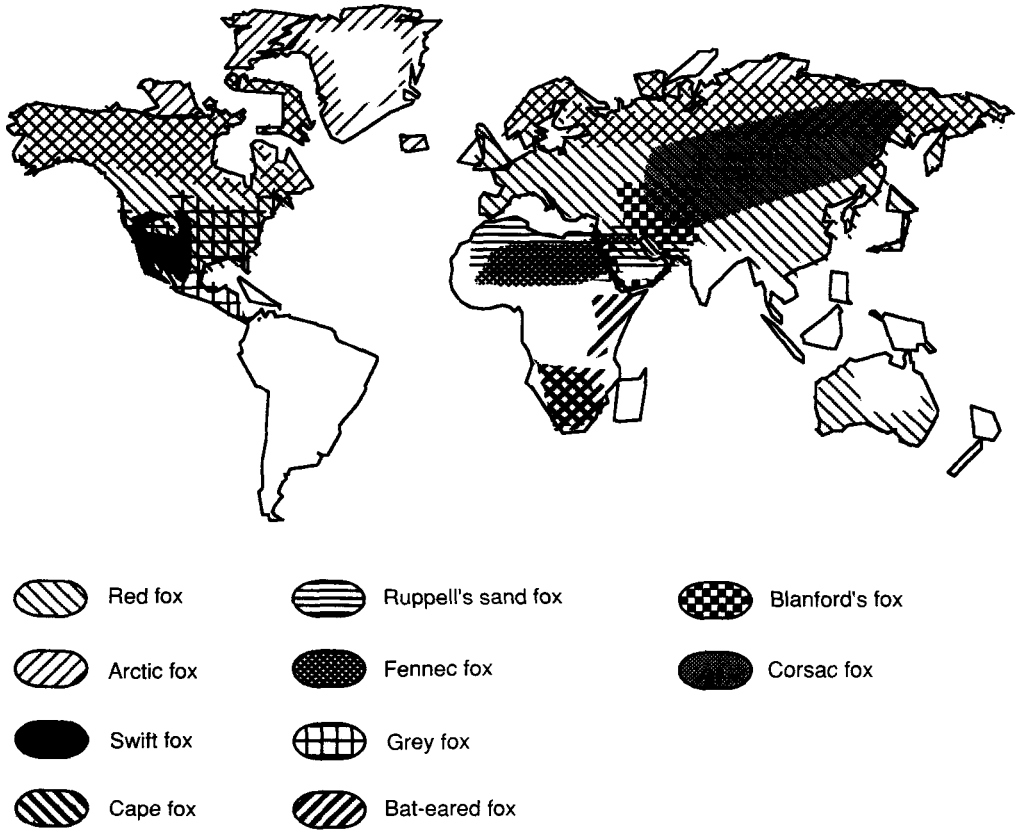

FIG. 1. Distribution map for the 10 fox-like canids that were examined.

Restriction fragment and site analysis

DNA was extracted by phenol-chloroform after overnight treatment with proteinase K at 65° C (Maniatis, Fritsch & Sanbrook, 1989). The following 19 restriction enzymes: *Ava* I, *Bam* HI, *Bcl* I, *Bgl* I, *Bgl* II, *Bst* EII, *Bst* XI, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hin*d III, *Hpa* I, *Sma* I, *Sst* II, *Stu* I, *Xba* I, *Xho* I and *Xmn* I were used separately to digest samples of approximately 10 μg of DNA from each individual. The digested DNA was separated electrophoretically on a 1% agarose gel, transferred to nylon membranes, and hybridized with the entire mtDNA genome cloned from a domestic dog [*Hin*d III digest of isolated dog mtDNA yields 3 fragments, approximately a 1kb fragment was cloned into pUC19 (BRL), and the other 2 fragments cloned into a single Lambda EMBL3 (Stratagene) vector]. Mitochondrial DNA fragments were visualized by autoradiography and identity was determined by comigration of fragments.

Mitochondrial DNA genotypes were constructed from composite restriction-fragment patterns on all 19 enzymes (Lehman *et al.*, 1991). Presence-absence of restriction sites was deduced from the pattern of fragment loss or gain among genotypes (Lansman *et al.*, 1983; Wayne *et al.*, 1989*b*; Lehman *et al.*, 1991). Such reconstructions may be accurate if genotypes usually differ by the loss or gain of single restriction sites but sometimes we needed to infer loss or gain of more than one site from the apparent size distribution of restriction fragments. In such cases, we assumed that the minimum number of restriction site changes had occurred. Because of this uncertainty, phylogenetic trees were constructed from both the matrix of shared

restriction sites and restriction fragments. The latter does not require inference of restriction site change as fragment identities are observed directly on autoradiograms. Rooting of trees was complicated by the lack of shared fragments between the desired outgroup (grey wolf) and the fox-like canids. Thus, we used the grey fox (*Urocyon cinereoargenteus*) as the outgroup in the restriction fragment and site analysis as it shared greater fragment homology with the other fox-like canids, and appeared to be a reasonable outgroup based on past molecular studies (Wayne & O'Brien, 1987; Wayne *et al.*, 1987*a*, 1989*a*). Presence-absence matrices of restriction sites and restriction fragments were analysed by maximum parsimony using the branch-and-bound algorithm of PAUP version 3·0 for the Apple Macintosh (Swofford, 1989). The percentage nucleotide sequence divergence between genotypes and its standard error were calculated by the restriction-site method of Nei & Li (1979) with modifications by Nei & Tajima (1983).

Cytochrome b sequencing

Two universal primers (H15149: Kocher *et al.*, 1989; L14725: Meyer & Wilson, 1990) were used to amplify a 402 bp segment of the mitochondrial cytochrome *b* gene by the polymerase chain reaction (PCR) method. A double-stranded sequence was amplified first and used in a second PCR reaction to generate a single-stranded template by the unbalanced primer method (Gyllensten & Erlich, 1988). Each PCR reaction mixture contained approximately 10 ng of genomic DNA; 1 mM dNTP mix in a reaction buffer of 50 mM KCl, 2·5 mM $MgCl_2$, 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 pmoles of each primer was used, and for the single-stranded amplification an unequal ratio of 25 to 0·25 pmoles was used. Thirty-five to 40 cycles of amplification were run in a programmable Perkin-Elmer Cetus DNA thermal cycler as follows: denaturation at 94° C for 1 min, annealing at 55° C for 2 min and extension at 72° C for 1 min 30 sec. The double-stranded reaction products were separated in a 3% Nusieve (FMC corporation, Rockland, MD) agarose gel in 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 100 microconcentrators (Amicon) and 7 μl of the concentrated single-stranded product was sequenced using the limited primer in the second PCR (Sanger & Coulson, 1975) and a Sequenase kit (US Biomedical).

We used 2 methods to reconstruct phylogenies from sequence data, with the grey wolf, *Canis lupus*, being used as an outgroup to root each tree. First, we used unweighed maximum parsimony with the branch-and-bound algorithm of PAUP version 3·0 for the Apple Macintosh (Swofford, 1989). Majority rule consensus trees were generated from the group of the most parsimonious trees (see below). One thousand bootstrapping replicates (Felsenstein, 1985) were done to determine the statistical confidence of each node. Because the frequency of mutation varies by position and type of mutation (transition/transversion; Brown, 1985; Irwin, Kocher & Wilson, 1991), we used maximum-likelihood analysis in the PHYLIP program (Version 3·2 adapted for the Apple Macintosh; Felsenstein, 1989) to take account of these biases. This maximum-likelihood analysis allows for unequal expected frequencies of the 4 bases, unequal rates of change of transitions and transversions, and different rates of change according to codon position or region of DNA. We used the empirically determined frequencies of nucleotides and an average transition/transversion ratio determined by pairwise comparisons of all taxa. To calculate the standard error of sequence divergence estimates we used the approach of Nei *et al.* (1985).

Results

The between species sequence divergence based on shared restriction sites varies from $0{\cdot}24 \pm 0{\cdot}23\%$ between arctic fox (Ala) and swift fox (Vve(Co)) to $14{\cdot}04 \pm 1{\cdot}02\%$ between Blanford's fox (Vca) and grey fox (Uci) (Table II). Average divergence between the grey fox and other *Vulpes*-like canids ($12{\cdot}0 \pm 1{\cdot}1\%$) are larger than those between foxes within the latter group

TABLE II

Percentage sequence divergence based on shared restriction sites (above diagonal) and shared restriction fragments (below diagonal)

Species[a]	Uci	Fze	Vru	Vve(Co)	Vve(Nv)	Vvu(Ca)	Vvu(SA1)	Vvu(SA2)	Vch	Vco	Ala	Vca
Uci	*	13·69	12·19	11·19	11·07	10·48	11·67	11·67	12·53	12·23	10·95	14·04
Fze	9·30	*	9·38	9·44	9·73	10·03	10·25	10·25	10·82	13·64	9·61	8·69
Vru	10·00	6·22	*	5·28	5·49	2·61	2·85	3·13	8·43	6·63	5·37	12·37
Vve(Co)	9·40	6·07	5·78	*	0·35	5·84	6·08	6·08	8·95	8·35	0·24	12·34
Vve(Nv)	8·84	5·97	5·44	0·33	*	5·73	5·96	5·96	9·26	8·22	0·60	12·70
Vvu(Ca)	7·19	6·69	2·42	5·04	4·95	*	0·97	1·22	7·83	5·40	5·61	11·66
Vvu(SA1)	8·68	6·94	2·51	4·99	4·90	0·68	*	0·23	8·09	6·02	5·84	11·89
Vvu(SA2)	8·26	6·94	2·97	4·99	4·90	0·98	0·26	*	8·51	6·38	5·84	11·89
Vch	8·75	8·79	7·28	7·69	7·59	7·14	7·09	7·44	*	9·68	8·69	12·68
Vco	8·41	8·46	5·04	7·11	7·34	3·96	3·91	4·11	6·82	*	8·09	12·41
Ala	8·02	5·97	4·76	0·51	0·61	5·63	5·35	5·35	7·59	6·12	*	12·59
Vca	11·12	6·69	10·15	8·02	8·28	8·19	8·14	8·14	9·45	7·54	9·06	*

[a] Uci = *Urocyon cinereoargenteus*, Fze = *Fennecus zerda*, Vru = *Vulpes ruppelli*, Vve (Co) = *Vulpes velox* from Colorado, Vve (Nv) = *Vulpes velox* from Nevada, Vvu(Ca) = *Vulpes vulpes* from California, Vvu(SA1) = *Vulpes vulpes* from Saudi Arabia (genotype #1), Vvu(SA2) = *Vulpes vulpes* from Saudi Arabia (genotype #2), Vch = *Vulpes chama*, Vco = *Vulpes corsac*, Ala = *Alopex lagopus*, Vca = *Vulpes cana*

(7·5 ± 3·6%). The within species values range from an average of 0·81 ± 0·51% for the red fox (Vvu) to 0·35 ± 0·28% for the swift fox (Vve). Sequence divergence values for the restriction fragment analysis are similar to the values for the restriction site analysis and are significantly associated according to a permutation test (Table II, Fig. 2a, $r = 0{\cdot}93$, $P < 0{\cdot}01$; Dietz, 1983). However, whereas sequence data from 402 bp of the cytochrome *b* gene show similar divergence patterns to that of the restriction site and fragment data, the magnitude of cytochrome *b* sequence divergence values are generally greater than the corresponding values for the fragment or site data (Table III). These values range from 22·0 ± 2·3% between bat-eared fox (Ome) and Corsac fox (Vco) to 3·7 ± 0·9% between swift fox and arctic fox (Ala). Within the fox-like canids, excluding the bat-eared fox and grey fox, the average sequence divergence is 14·3 ± 3·1%, a value approximately twice as large as that based on restriction site data. Other values of sequence divergence, such as that between the swift fox and arctic fox, are nearly an order of magnitude larger (3·7% vs. 0·4%). However, the correlation between the cytochrome *b* sequence estimates and those based on restriction fragment or restriction site data is significant by a permutation test ($r = 0{\cdot}69$, $P < 0{\cdot}01$ and $r = 0{\cdot}62$, $P < 0{\cdot}05$, respectively) and approximately linear (Fig. 2b, c).

Phylogenetic trees based on shared restriction fragments, restriction sites and cytochrome *b* sequence are topologically very similar (Fig. 3). The following elements are consistent among the four trees: (1) the arctic fox and swift fox are closely related sister taxa. Bootstrap replicates indicate this is a highly significant association; (2) Blanford's fox and the fennec fox are always associated as sister taxa, however, the sequence divergence between these two taxa is sizeable (7%–10%, Tables II and III); (3) in the cytochrome *b* sequence analysis, the genera *Alopex, Fennecus* and *Vulpes* form a monophyletic group that does not include the bat-eared fox (*Otocyon*) or the grey fox (*Urocyon*), suggesting a closer kinship of the first three genera and; (4) the Cape fox has a basal position in this monophyletic group. The trees are not consistent with respect to the relationships of Ruppell's sand fox, the Corsac fox and the red fox. In all of the trees these taxa are grouped with each other and the swift and arctic fox but the specific affinities within this clade are not clear.

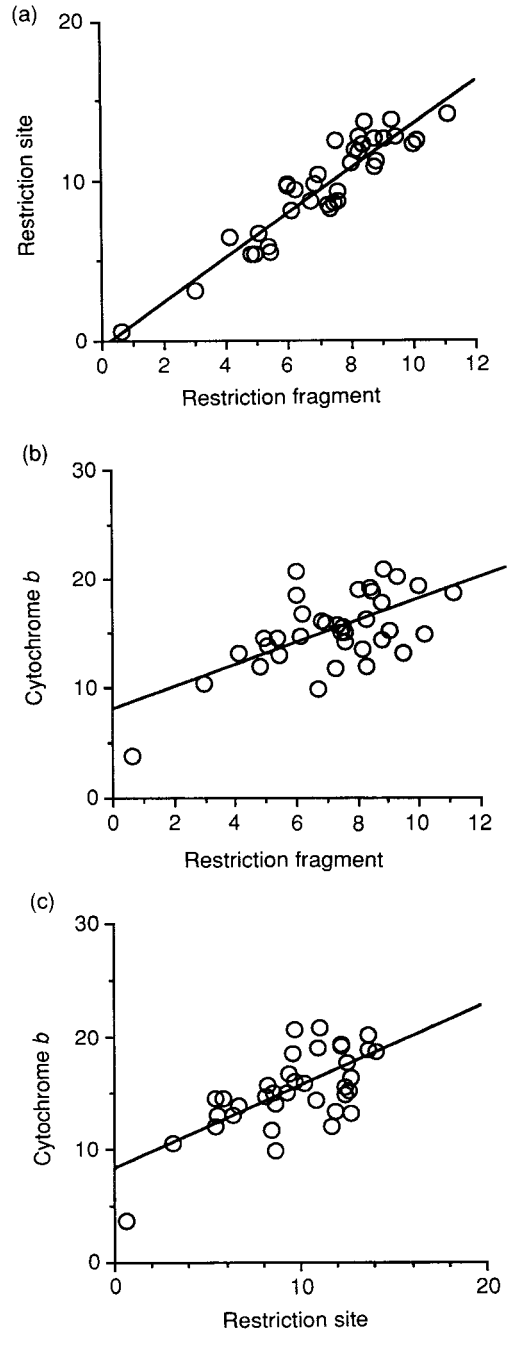

FIG. 2. The relationships between percentage sequence divergence estimates based on shared restriction fragments, shared restriction sites, and cytochrome *b* sequence data. Lines determined by least-square linear regression fit to data: (a) $y=1{\cdot}38x-0{\cdot}30$; (b) $y=1{\cdot}01x+7{\cdot}95$; (c) $y=0{\cdot}74x+8{\cdot}04$.

TABLE III

Maximum-likelihood cytochrome b sequence divergence matrix (above diagonal) and number of base substitutions (below diagonal). See Table II for species codes

Species[a]	Uci	Ome	Vvu	Vca	Fze	Vch	Vru	Vco	Vve	Ala	Clu
Uci	*	15·99	11·96	18·69	20·13	17·72	19·31	19·10	20·85	19·00	15·87
Ome	53	*	16·91	15·70	16·56	14·39	16·83	22·04	20·19	19·27	16·15
Vvu	43	56	*	13·37	15·80	15·00	10·42	13·07	14·49	14·42	17·81
Vca	62	52	46	*	9·85	13·15	14·79	15·54	16·25	15·17	18·07
Fze	57	48	46	30	*	14·36	16·70	18·78	20·66	18·44	20·11
Vch	59	48	51	45	42	*	11·75	16·01	15·07	14·07	16·37
Vru	61	54	35	48	48	39	*	13·76	12·96	11·99	21·04
Vco	59	67	42	49	52	51	44	*	15·71	14·67	21·34
Vve	66	64	48	53	57	50	42	49	*	3·72	21·14
Ala	61	62	48	50	53	47	40	47	13	*	19·64
Clu	54	53	60	59	57	54	65	64	66	62	*

[a]Ome = *Otocyon megalotis*, Clu = *Canis lupus* and for other species codes see Table II

Discussion

The correspondence of sequence divergence measures

In this study, we estimate mtDNA sequence divergence using restriction fragment and restriction site data as well as direct sequence data from 402 bp of the cytochrome *b* gene. Whereas the former two approaches yield estimates of sequence divergence which are very similar to each other, the cytochrome *b* sequence data provides much higher divergence estimates. These values range from 1·4 to 9 times the equivalent values from restriction site or restriction fragment data. The discrepancy is greatest for divergence values among closely related taxa (Tables II and III, Fig. 2). Because restriction site variation appears to fall uniformly throughout the mtDNA genome (Cann, Brown & Wilson, 1984), the higher sequence divergence values for cytochrome *b* suggests it has a higher than average rate of sequence evolution. This result is consistent with past studies of mammalian cytochrome *b* sequence variation (Brown, 1985). The greater discrepancy in sequence divergence estimates among recently diverged taxa may reflect the increasing saturation of variable sites over time such that divergence values for restriction data and cytochrome *b* sequence data eventually approach a similar asymptote (Irwin *et al.*, 1991).

Evolution of the New World desert foxes

The majority of past studies based on morphological data have indicated that the arctic fox warrants generic distinction (Huxley, 1880; Mivart, 1890; Simpson, 1945; Clutton-Brock *et al.*, 1976; van Gelder, 1978). The most recent study of morphological similarity of cranial, dental and external characteristics advocated placing *Alopex* in a separate genus, whereas other fox-like canids including *Urocyon* were placed in *Vulpes* (Clutton-Brock *et al.*, 1976). This view is somewhat reaffirmed by van Gelder (1978) who demoted all fox-like canids, except *Otocyon* and *Alopex* to a single subgenus of *Canis*. Independent genetic data now strongly contradict the generic distinction of *Alopex*. The arctic fox and swift fox share a karyotype unique among the Canidae and the allozyme similarity between them is higher than between other sampled fox-like canids (Table I; Wayne & O'Brien, 1987; Wayne *et al.*, 1987*b*). DNA hybridization data also suggest the

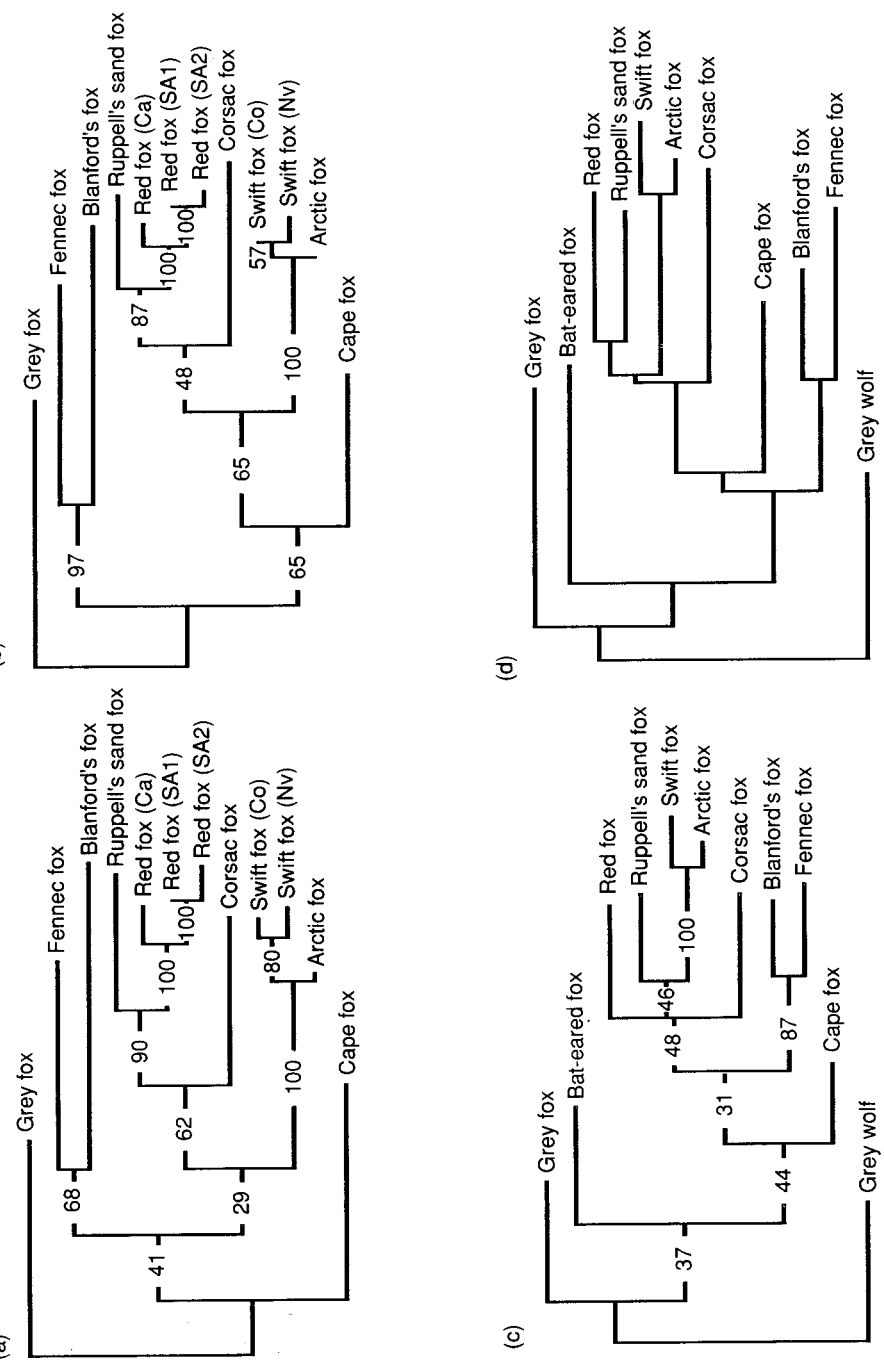

FIG. 3. (a) The most parsimonious tree based on presence-absence matrix of shared restriction fragments (tree length = 387 and overall consistency index = 0·67). (b) One of two most parsimonious trees based on the presence-absence matrix of restriction sites (tree length = 250 and overall consistency index = 0·69). The second tree (not shown) differed only in placing the arctic fox as a sister taxon to the swift fox from Colorado. (c) Majority rule consensus tree derived for the seven most parsimonious trees based on phylogenetic analysis of 402 bp of cytochrome *b* sequence (tree length = 288 and overall consistency index = 0·64). (d) Phylogenetic tree generated by maximum-likelihood method based on 402 bp of cytochrome *b* sequence (see text). All branch lengths are significantly different from zero ($P < 0{\cdot}05$) except for the internode connecting the Corsac fox with other foxes. Numbers in internodes refer to the percentage trees out of 1000 bootstrap replications with the shown bifurcation (Felsenstein, 1985).

arctic fox is as similar to species of *Vulpes* as such species are to each other (Wayne *et al.*, 1989*a*). The mtDNA data provide independent confirmation of this view, and further suggest that the arctic fox and swift fox are very closely related. In fact, sequence divergence values between the two subspecies of swift fox in Table II are similar to values between these taxa and the arctic fox.

The discrepancy between morphological and genetic measures may reflect the profound habitat differences between that of swift foxes, which live in arid regions of the American south-west, and that of the arctic fox, which has a circumpolar distribution. Arctic and arid conditions impose dramatically different selective regimes which may have accelerated the morphological divergence apparent between the two taxa. In contrast, molecular divergence, if due primarily to selectively neutral or nearly-neutral changes, may in this case be a more accurate indicator of separation time. Indeed, the value of mtDNA sequence divergence based on restriction site data between the arctic fox and swift fox is small, approximately 0·5%, suggesting a divergence time of 250,000 years before present if a conversion of 2% per million years is used (Shields & Wilson, 1987). The fossil appearance of these two taxa in the mid-Pleistocene suggests a slightly earlier time of origin (Kurtén & Anderson, 1980). The appearance of the arctic fox in the European Steinheimian (400,000–500,000 years ago) is more recent than that of the swift fox which appeared in North America during the late Irvingtonian (> 500,000 years ago; Savage & Russell, 1983). The historical geographic range of the swift fox extends into southern Canada and is nearly contiguous with the southern-most part of the arctic fox range in North America (Fig. 1). If we assume that the swift fox was ancestral to the arctic fox, then conceivably the evolution of the arctic fox from a swift fox-like ancestor may have taken place as glaciations became common and extensive toward the end of the Pleistocene. Arid-land foxes are somewhat preadapted to life in the tundra because the tundra is essentially a desert environment. Tundra conditions that became more prevalent during glacial periods may have intensified selection for cold climate adaptations in some arid-land populations of swift foxes. Similarly, the evolution of the polar bear, *Ursus maritimus*, from its progenitor, the brown bear, *Ursus arctos*, may reflect the changing conditions during the late Pleistocene. These two species are genetically very close (Goldman, Giri & O'Brien, 1989; Shields & Kocher, 1991) and the polar bear has a recent fossil record (< 10,000 years ago), whereas the brown bear has a fossil record extending into the mid-Pleistocene (Kurtén, 1964; Kurtén & Anderson, 1980). These two examples suggest that the recent glacial periods promoted the origination of terrestrial mammals adapted for living on snow and ice which were derived from forms whose geographic range included warmer southern regions.

Evolution of the Old World desert foxes

Three foxes are common in the desert regions of the middle East and northern Africa: Blanford's fox, *Vulpes cana*; the fennec fox, *Fennecus zerda*; and Ruppell's sand fox, *Vulpes ruppelli*. The phylogenetic analyses of mtDNA restriction site, restriction fragment and cytochrome *b* sequence data show that the former two taxa are sister species distinct from the other fox-like canids (Fig. 3). Thus, they define a taxonomic grouping that has not been previously recognized. However, based on restriction site data, the sequence divergence between the fennec fox (Fze) and Blanford's fox (Vca) is large, approximately 8·5%, indicating an ancient divergence time of about 4–4·5 million years ago (Table II). This divergence is nearly coincident with the appearance of desert regions in the Middle East and northern Africa (Wickens, 1984), and suggests that a fox-like progenitor entered these regions and diversified into two lineages. The fennec occupies a habitat in shifting sand dune environments whereas Blanford's fox is restricted to steep

rocky slopes. Each species shows distinct morphological adaptations for these habitats. For example, Blanford's fox has hairless feet adapted for climbing on bare rock and the fennec has furred pads for locomotion on shifting sand (Mendelssohn *et al.*, 1987). Whereas other fox species usually occupy a range of habitats, the fennec and the Blanford's foxes, which are the smallest of all canids, show a strong affinity to a single, specific habitat. Their small size (Table I) may be associated with their specialization for the more arid and poorer quality habitats of the Arabian desert (Case, 1978; Gittleman, 1985). The more distal divergence of Ruppell's fox from other *Vulpes* species suggests that it may have entered desert regions more recently. Ruppell's fox has been successful at establishing itself in more vegetated environments of desert regions and may even be replaced near human developments by the red fox, *Vulpes vulpes* (Lindsay & Macdonald, 1986). Both species are more than twice as large as Blanford's fox or the fennec, and do not persist in the poorer quality habitats where fennec and Blanford's foxes are common (Mendelssohn *et al.*, 1987; Harrison & Bates, 1991).

Stable long-term co-existence of morphologically similar canids with different habitat preferences was also found in African jackals (Wayne *et al.*, 1989*b*, 1990). In East Africa, three jackal species co-exist: the golden jackal, *Canis aureus*; the black-backed jackal, *C. mesomelas*; and the side-striped jackal, *C. adustus*. As in the desert foxes, these three species are not closely-related, thus their morphological similarity is not due to a recent evolutionary divergence. Moreover, analogous to the desert foxes, their habitat requirements and activity patterns are slightly different (Fuller *et al.*, 1989). Like Ruppell's fox, the golden jackal appears to be a more recent addition to the fauna, is derived from a different canid stock than the other jackal species, and occupies a separate habitat within the Serengeti ecosystem. Such potential examples of ecological character displacement may be common in canids (Dayan *et al.*, 1989; Wayne *et al.*, 1989*b*).

Relationships of the Cape fox

The Cape fox, *Vulpes chama*, is distantly related to other *Vulpes* species and appears to have diverged early in the history of the group. This finding is consistent with past allozyme results that show *Vulpes chama* to be distantly related to other species in *Vulpes*, *Fennecus* and *Alopex* (Wayne & O'Brien, 1987). Clutton-Brock *et al.* (1976) indicated that the Cape fox is the most typical member of its genus with respect to cranial measurements and has close affinities to the Bengal fox, *Vulpes bengalensis*. Our results suggest that the Cape fox may be an early offshoot of the genus and thus may have retained primitive morphological characters that are potentially held in common by several taxa. Unfortunately, we were not able to obtain samples of the Bengal fox so we could not examine relationships between it and the Cape fox.

The early divergence of the Cape fox from other *Vulpes* species may reflect a relatively long isolation in southern Africa. The Cape fox is found only in the southern tip of Africa (Fig. 1) and no other *Vulpes* species appear south of the Sahara. The range of sequence divergence values between the Cape fox and other *Vulpes* species suggests an ancient immigration of *Vulpes*-like canids into southern Africa about 4–5 million years before present. An ancient immigration of canids into Africa is also supported by the degree of divergence seen between black-backed jackal genotypes (Wayne *et al.*, 1990). Unfortunately, the fossil record of the Cape fox is poor; the earliest specimens are approximately 1–2 million years old.

Relationships of other ***Vulpes*** *species*

The Corsac, Ruppell's sand, red, swift and arctic foxes define a consistent clade (Fig. 3). The specific branching pattern of these taxa is unresolved. Phenetic analysis by Clutton-Brock *et al.* (1976) did not show a similar grouping of these taxa. However, we were not able to analyse three species, *V. bengalensis*, *V. pallida* and *V. ferrilata*, and if these taxa were included the coherency and relationships of taxa in this group might change. Our results do suggest that desert-adapted forms have evolved independently at least twice, once in the form of the fennec and Blanford's fox and more recently as the swift and the Ruppell's sand foxes.

Relationships of the grey fox and bat-eared fox

Our phylogenetic analysis of mtDNA cytochrome *b* sequence data clearly indicates that the bat-eared fox and the grey fox are distant relatives of other fox-like taxa. This result was supported by older studies of the Canidae (Huxley, 1880; Mivart, 1890; Simpson, 1945), but recently Clutton-Brock *et al.* (1976) and van Gelder (1978) suggested grouping *Urocyon* with *Vulpes*. Past molecular and chromosome studies strongly suggest that the grey fox represents a distinct evolutionary lineage which, although fox-like in conformation, branched early within the Canidae (Wayne *et al.*, 1987*a*, *b*; Wayne & O'Brien, 1987; Wayne *et al.*, 1989*a*). The fossil record of the grey fox also extends to approximately 4–6 million years ago (Kurtén & Anderson, 1980) which is consistent with an early divergence of this species from other fox-like canids. The fossil record of the bat-eared fox is sparse and the first specimens are known from the Pliocene about 3 million years ago (Savage & Russell, 1983).

Taxonomic recommendations

Generic distinctions should be based on monophyletic groupings of taxa (Wiley, 1981). Our results suggest that a monophyletic grouping that includes taxa in the genera *Fennecus*, *Alopex* and *Vulpes* can be defined. To distinguish them as separate genera seems inconsistent relative to other generic divisions within the family. These taxa, based on arguments of priority, should be synonymized under the genus *Vulpes* (Clutton-Brock *et al.*, 1976). Clearly, *Alopex* should not be given separate generic status.

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