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Resolution of Recent Radiations Within Three Evolutionary Lineages of Felidae Using Mitochondrial Restriction Fragment Length Polymorphism Variation

Warren E. Johnson,[1,4] Peter A. Dratch,[2,3] Janice S. Martenson,[1] and Stephen J. O'Brien[1]

Patterns of mitochondrial restriction fragment length polymorphism (RFLP) variation were used to resolve more recent relationships among the species of the Felidae ocelot lineage, domestic cat lineage, and pantherine lineage. Twenty-five of 28 restriction enzymes revealed site variation in at least 1 of 21 cat species. The ocelot lineage was resolved into three separate sister-taxa groups: Geoffroy's cat (*Oncifelis geoffroyi*) and kodkod (*O. guigna*), ocelot (*Leopardus pardalis*) and margay (*L. wiedii*), and pampas cat (*Lynchailurus colocolo*) and most of the tigrina samples (*Leopardus tigrina*). Within the domestic cat lineage, domestic cat (*Felis catus*), European wild cat (*F. silvestris*), and African wild cat (*F. libyca*) formed a monophyletic trichotomy, which was joined with sand cat (*F. margarita*) to a common ancestor. Jungle cat (*F. chaus*) and black-footed cat (*F. nigripes*) mtDNAs diverged earlier than those of the other domestic cat lineage species and are less closely related. Within the pantherine lineage, phylogenetic analysis identified two distinct groups, uniting lion (*P. leo*) with leopard (*P. pardus*) and tiger (*P. tigris*) with snow leopard (*P. uncia*).

KEY WORDS: Felidae; mitochondrial DNA; phylogenetic reconstruction; restriction fragment length polymorphism.

INTRODUCTION

The family Felidae provides a diverse group of species with which to examine processes of evolution and resulting molecular genetic patterns. Dissimilar patterns of diversification, evolutionary history, and distribution make these species useful for characterizing genetic processes. Felid species have received a great deal of scientific and popular attention because of their charisma, important ecological roles, and conservation status due to habitat destruction and overhunting. Extensive descriptive information has accu-

[1] Laboratory of Viral Carcinogenesis, NCI–Frederick Cancer Research and Development Center, Frederick, Maryland 21702.
[2] Biological Carcinogenesis and Development Program, SAIC NCI–Frederick Cancer Research and Development Center, Frederick, Maryland 21702.
[3] Present address: National Fish and Wildlife Forensic Laboratory, 1490 East Main Street, Ashland, Oregon 97520.
[4] To whom correspondence should be addressed.

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mulated on their natural histories, morphology, behavior, reproduction, evolutionary history, and population genetic structure, which provides a rich basis for interpreting genetic data (Seidensticker and Lumpkin, 1991; Guggisberg, 1975; O'Brien, 1994a,b; O'Brien *et al.*, 1996).

The 36–38 extant felid species are divided into several clades or lineages (Collier and O'Brien, 1985; O'Brien, 1986; Salles, 1992; Wozencraft, 1993). The best described of these clades are the ocelot lineage, including the ocelot (*Leopardus pardalis*), margay (*L. wiedii*), tigrina (*L. tigrina*), pampas cat (*Lynchailurus colocolo*), Geoffroy's cat (*Oncifelis geoffroyi*), kodkod (*O. guigna*), and probably Andean mountain cat (*Oreailurus jacobita*); the domestic cat lineage, including the domestic cat (*Felis catus*), African wild cat (*F. libyca*), European wild cat (*F. silvestris*), sand cat (*F. margarita*), black-footed cat (*F. nigripes*), jungle cat (*F. chaus*), and Pallas cat (*Otocolobus manul*); and the *Panthera* genus of the pantherine lineage, composed of the lion (*Panthera leo*), jaguar (*P. onca*), tiger (*P. tigris*), snow leopard (*P. uncia*), leopard (*P. pardus*), and possibly the clouded leopard (*Neofelis nebulosa*) (Wayne *et al.*, 1989; scientific nomenclature follows Ewer, 1973). These groups were defined on the basis of comparative karyology (Wurster-Hill and Centerwall, 1982; Modi and O'Brien, 1988), the presence of two felid endogenous retroviruses in domestic cats (Benveniste and Todaro, 1974; Benveniste *et al.*, 1975; Reeves and O'Brien, 1984), albumin immunological distance (Collier and O'Brien, 1985), allozyme electrophoresis (O'Brien *et al.*, 1987; Pecon Slattery *et al.*, 1994), and two-dimensional protein electrophoresis (Pecon Slattery *et al.*, 1994).

Each of these lineages exhibits contrasting patterns of phylogeographic histories. The ancestors of the ocelot lineage, made up of small spotted cats in Central and South America (2–18 kg), probably diverged from a precursor to modern Felidae 10–12 million years ago (MYA), but evolved recently and rapidly following the formation of the Panama landbridge between North and South America (<3 MYA) (Wayne *et al.*, 1989; Pecon Slattery *et al.*, 1994). The domestic cat lineage diverged from the other felids more recently (8–10 MYA) and is composed of small, morphologically similar cats which have differentiated from each other over a wide area of Africa, Europe, and Asia (Guggisberg, 1975; Seidensticker and Lumpkin, 1991). The pantherine lineage includes the large and midsize cat species which diverged over the last 5–7 MY. A more recent radiation of the pantherine lineage led to the *Panthera* genus. These are the great cats (15–300 kg) with almost worldwide distribution, but which have differentiated only recently (Neff, 1982; O'Brien *et al.*, 1987; Janczewski *et al.*, 1995). Although these three major groups have been well supported by a variety of methods, the evolutionary associations within the lineages remain unresolved.

The present study addresses the recent phylogenetic relationships among species within each of the three major lineages using mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLP). MtDNA has several traits which make it useful for phylogenetic analysis, including nearly complete maternal, clonal inheritance, a general lack of recombination, and a relatively rapid rate of evolution (Avise *et al.*, 1987; Brown, 1985; Wilson *et al.*, 1985; Avise, 1991, 1994). RFLP analysis has the advantage of rapidly sampling the entire mitochondrial genome.

Previous research with felids using mtDNA RFLP analysis has addressed a variety of taxonomic, phylogeographic, and population level questions in selected species.

MtDNA RFLP analysis of the endangered Florida panther subspecies (*Puma concolor coryi*) demonstrated genetic introgession of South American pumas into one of two extant Florida populations (O'Brien *et al.*, 1990). In an analysis of leopards sampled from around the world, mtDNA RFLP was used (1) to determine genetic distance and divergence dates among leopard populations, (2) to demonstrate an African origin for leopards, and (3) to address subspecies classification issues (Miththapala *et al.*, 1996). In cheetahs (*Acinonyx jubatus*), mtDNA RFLP was used to estimate levels of genetic variation and, combined with minisatellite data, revealed that cheetahs experienced a severe genetic bottleneck approximately 10,000 years ago (Menotti-Raymond and O'Brien, 1993). The present study provides the first comprehensive comparison of mtDNA restriction site divergence in felid species.

MATERIALS AND METHODS

Total genomic DNA was extracted, following standard methods described by Modi *et al.* (1987) and Sambrook *et al.* (1989), from frozen leukocytes, primary fibroblast cultures from skin biopsies, or frozen organs (liver, kidney, ovary) from several individuals of each of 21 felid species and a hyaenid, spotted hyena (*Crocuta crocuta*), which was used as the outgroup (Table I).

One microgram of DNA from each animal was digested with a panel of 28 restriction enzymes from LTI/BRL (Table II). Enzymes with more than one recognition site were chosen based on a preliminary screening. Digested samples were separated by electrophoresis on 1% agarose gels in TAE buffer (40 m*M* Tris, 20 m*M* sodium acetate, 1 m*M* EDTA at pH 7.2) for 16 hr at 70 V and 65 mA, then denatured, neutralized, and transferred by Southern blotting in 10× standard saline citrate (SSC) onto nylon membranes (UV Duralon, Stratagene). DNA was fixed onto membranes by UV cross-linking for 30 sec at 120,000 J (Stratalinker TM 1800 UV Crosslinker, Stratagene). Cloned domestic cat mtDNA (O'Brien *et al.*, 1990) was randomly primed (Random Prime Kit, Boehringer-Mannheim) with [^{32}P]dCTP (New England Nuclear) and hybridized to the membranes at 37°C for 16 hr in a solution of 50% formamide, 1 *M* NaCl, 10 m*M* EDTA, 50 m*M* PIPES (pH 6.4), 1% sodium dodecyl sulfate (SDS), 5× Denhardt's solution, and 200 mg of denatured salmon sperm DNA. Nonspecific radioactivity was removed from the membranes with three increasingly stringent SSC/SDS washes (to a final stringency of 0.2× SSC and 0.5% SDS). Membranes were blotted dry and hybridized; fragments were visualized by autoradiography (Fig. 1).

A molecular weight standard of *Bam*HI/*Eco*RI digest of adenovirus II DNA (IBI) labeled with [^{32}P]dATP was used to size fragments. DNA samples from total genomic DNA and from mtDNA isolated from organ tissue on a cesium chloride gradient (Sambrook *et al.*, 1989) were run separately for each individual of a tested species to distinguish fragments of mitochondrial sequences which may have become incorporated into the nuclear genome (*Numt*) of some cat species (Lopez *et al.*, 1994).

Estimated sizes of fragments were summed for general concordance with domestic cat mitochondrial DNA, which has a length of 17 kb, disregarding putative *Numt* fragments (Lopez *et al.*, 1996). Restriction patterns (band patterns) for each enzyme were compared, and individuals were scored for presence or absence of restriction sites (frag-

Table I. Identification Code, Sex, and Source for Each Sample (Scientific Names Follow Ewer, 1973)

Species	Sample	Sex	Source
Ocelot lineage			
Ocelot (*Leopardus pardalis*)	Lpa3	M	Henry Doorly Zoo, Nebraska
	Lpa6	M	Blijdorp Zoo, Netherlands
	Lpa7	F	Octagon Wildlife Park, Florida
Margay (*Leopardus wiedii*)	Lwi1	M	Carnivore Preserve Trust, North Carolina
	Lwi8	F	Blijdorp Zoo, Netherlands
	Lwi12	F	Brookfield Zoo, Illinois
Tigrina (*Leopardus tigrina*)	Lti3	F	Cincinnati Zoo, Ohio
	Lti5	F	P. Zool. et Botan. de Mulhouse, France
	Lti8	U	SOS Care, California
	Lti65	M	Cincinnati Zoo, Ohio
Pampas cat (*Lynchailurus colocolo*)	Lco4	F	Zoológico Nacional de Chile
Geoffroy's cat (*Oncifelis geoffroyi*)	Oge66	M	Carnivore Preserve Trust, North Carolina
	Oge3	M	Blijdorp Zoo, Netherlands
	Oge8	F	National Zoological Park, Washington, D.C.
	Oge11	M	Washington State University
Kodkod (*Oncifelis guigna*)	Ogu2	F	Zoológico Nacional de Chile
	Ogu3	F	Zoológico Nacional de Chile
Domestic cat lineage			
Domestic cat (*Felis catus*)	Fca84	F	NIH Animal Center, Maryland
African wild cat (*Felis libica*)	Fli1	M	Kruger Park, South Africa
European wild cat (*Felis silvestris*)	Fsi7	M	ISEC, Ohio
	Fsi10	F	San Antonio Zoological Gardens, Texas
	Fsi12	F	Zoologischer Garten Koln, Germany
Sand cat (*Felis margarita*)	Fma5	M	Brookfield Zoo, Illinois
	Fma10	M	Living Desert, California
	Fma11	M	Washington Park Zoo, Oregon
Black-footed cat (*Felis nigripes*)	Fni3	U	Meloy Laboratories, Virginia
	Fni6	M	San Diego Zoo, California
	Fni7	F	San Diego Zoo, California
Jungle cat (*Felis chaus*)	Fch2	M	Blijdorp Zoo, Netherlands
	Fch4	U	Meloy Laboratories, Virginia
Pallas cat (*Otocolobus manul*)	Oma4	F	Baltimore Zoo, Maryland
	Oma10	F	Brookfield Zoo, Illinois
Pantherine lineage			
Leopard (*Panthera pardus*)	Ppa5	F	Henry Doorly Zoo, Nebraska
	Ppa6	F	Henry Doorly Zoo, Nebraska
	Ppa20	U	Minnesota Zoological Gardens
	Ppa21	U	Minnesota Zoological Gardens
	Ppa30	F	Lincoln Park Zoo, Illinois
Lion (*Panthera leo*)	Ple7	F	National Zoological Park, Wash. D.C.
	Ple13	M	National Zoological Park, Wash. D.C.
	Ple23	M	Wildlife Safari Park, Oregon
	Ple24	F	National Zoological Park, Wash. D.C.
	Ple105	U	Woodland Park Zoo, Washington
Jaguar (*Panthera onca*)	Pon1	F	Carnivore Preserve Trust, North Carolina
	Pon9	U	Johannesburg Zool. Garden, S. Africa
Snow leopard (*Panthera uncia*)	Pun4	M	Detroit Zoo, Michigan
	Pun9	M	Calgary Zoo-Botanical Garden, Canada
	Pun10	F	New York Zoological Park
Tiger (*Panthera tigris*)	Pti2	M	Carnivore Preserve Trust, North Carolina
	Pti48	M	Minnesota Zoological Garden
	Pti65	M	Philadelphia Zool. Garden, Pennsylvania
	Pti66	F	Stone Zoo, Massachusetts
	Pti69	M	New York Zoological Park
	Pti76	M	Rare Feline Breeding Colony, California
	Pti77	M	Knoxville Zoological Park, Tennessee

Table I. Continued

Species	Sample	Sex	Source
	Pti81	F	San Diego Zoo, California
	Pti82	M	San Diego Zoo, California
	Pti83	F	San Diego Zoo, California
Clouded leopard (*Neofelis nebulosa*)	Nne9	F	National Zoological Park, Wash. D.C.
	Nne19	F	Minnesota Zoological Garden
	Nne22	F	NZP/Conservation Research Center, Virginia
Jaguarundi (*Herpailurus yagouaroundi*)	Hya8	M	San Diego Zoo, California
Cheetah (*Acinonyx jubatus*)	Aju70	F	St. Louis Zoological Park, Illinois
	Aju96	F	Wildlife Safari, Oregon
	Aju227	U	Nairobi Orphanage, Kenya
	Aju254	M	St. Louis Zoological Park
Outgroup			
Spotted hyena (*Crocuta crocuta*)	Ccr2	F	Henry Doorly Zoo, Nebraska

Table II. Polymorphic Restriction Enzymes Within Felid Species[a]

Species	Code	Sample size	Number of haplotypes	Polymorphic restriction enzymes
Leopardus pardalis	Lpa	3	3	*Acc*I, *Ava*I, *Ava*II, *Bcl*I, *Cla*I, *Hin*dIII, *Nde*I, *Stu*I, *Xba*I
Leopardus wiedii	Lwi	3	3	*Ava*I, *Ava*II, *Bam*Hi, *Bcl*I, *Eco*RI, *Hin*cII, *Hin*dIII, *Hpa*I, *Nco*I, *Nde*I, *Sst*I
Leopardus tigrina	Lti	4	4	*Acc*I, *Ava*I, *Ava*II, *Bam*HI, *Bgl*I, *Bst*UI, *Bst*EII, *Cla*I, *DRa*I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Hpa*I, *Nco*I, *Nde*I, *Stu*I, *Xba*I
Oncifelis geoffroyi	Oge	4	4	*Acc*I, *Apa*I, *Ava*II, *Bgl*II, *Bst*UI, *Bst*EII, *Cla*I, *Dra*I, *Nde*I, *Sst*I, *Stu*I
Oncifelis guigna	Ogu	2	2	*Apa*I, *Nco*I
Felis silvestris	Fsi	3	3	*Apa*I, *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*I, *Bst*UI, *Cla*I, *Eco*RI, *Nco*I, *Nde*I, *Sma*I, *Sst*I, *Xba*I
Felis margarita	Fma	3	1	
Felis nigripes	Fni	3	2	*Apa*I, *Ava*I, *Ava*II, *Bst*EII, *Cla*I, *Nco*I, *Sma*I, *Sst*I, *Xba*I
Felis chaus	Fch	2	1	
Otocolobus manul	Oma	2	1	
Panthera pardus	Ppa	5	4	*Ava*II, *Ava*I*, *Bst*UI, *Cla*I*, *Eco*RV*, *Hin*cII*, *Hin*dIII*, *Hpa*I*, *Nco*I, *Pst*I, *Xba*I
Panthera leo	Ple	5	3	*Ava*II*, *Bam*HI*, *Bgl*I*, *Pst*I
Panthera onca	Pon	2	2	*Ava*II, *Bst*UI, *Dra*I, *Hin*dIII, *Kpn*I, *Nco*I, *Sst*I
Panthera uncia	Pun	3	2	EcoRV
Panthera tigris	Pti	10	1	
Neofelis nebulosa	Nne	3	1	
Acinonyx jubatus	Aju	4	1	

[a] Asterisks refer to restriction enzymes known to be polymorphic in this species from other studies. Cheetahs (*A. jubatus*) and tigers (*P. tigris*) were monomorphic for all 28 restriction enzymes.

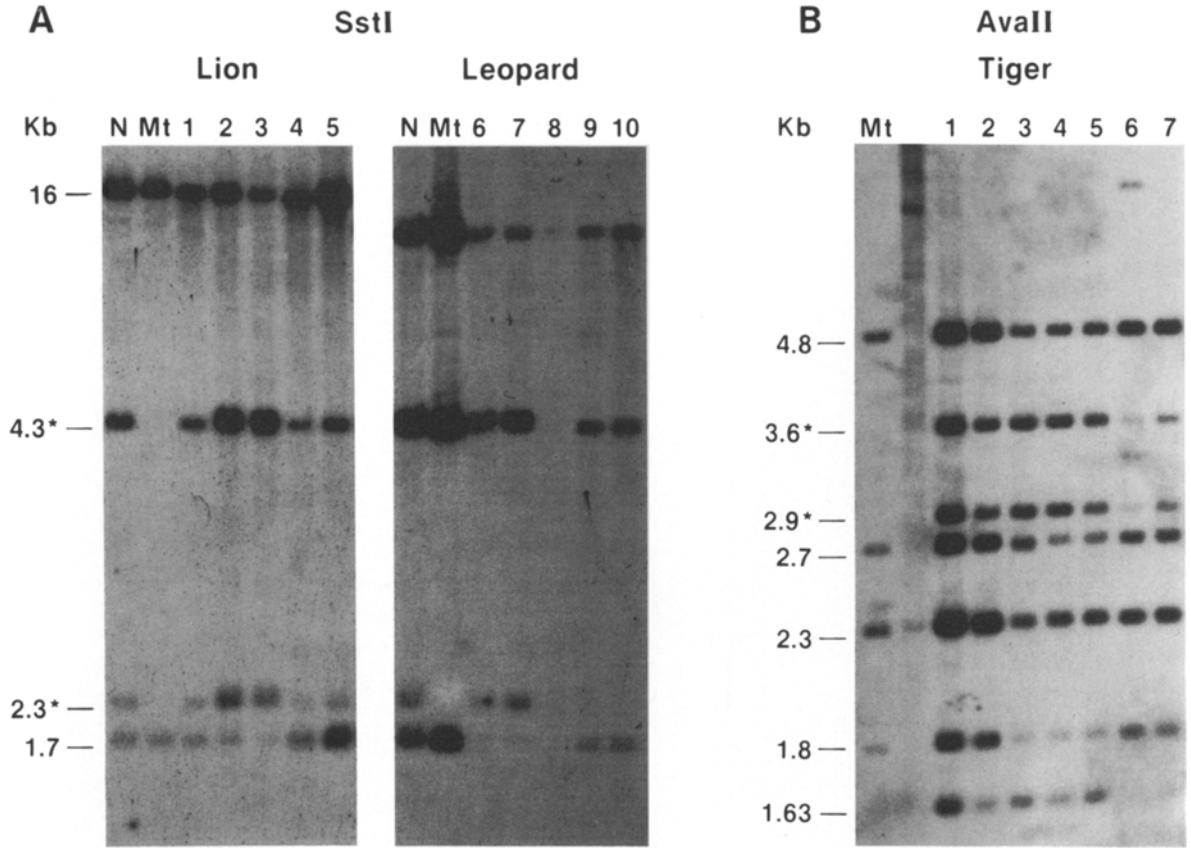


Fig. 1. MtDNA restriction patterns observed in fractionated and genomic DNA preparations of lion, leopard, and tiger. Fractions were nuclear DNA (N) and cytoplasmic DNA (Mt). The asterisks mark the nucleus-specific mtDNA fragments [see text and Lopez *et al.* (1994)]. Band sizes are in kilobase pairs (kb). A molecular weight standard of a *Bam*HI/*Eco*RI digest of adenovirus II DNA (IBI) labeled with [^{32}P]dCTP was used to size fragments. (A) Restriction patterns of hybridization in a *Sst*I digest of genomic DNA with five distinct lions and leopards. *Numt* bands are 4.3 and 2.3 kb in lion and 2.3 kb in leopard. (B) Restriction patterns of hybridization in an *Ava*II digest of genomic DNA with eight distinct tigers. *Numt* bands are 3.6 and 2.9 kb.

ments of equal sizes). Individual haplotypes were compiled from patterns of restriction sites across all the enzymes.

To facilitate comparison of shared fragments for each enzyme, all individuals of the ocelot lineage were run on one gel and all individuals of the domestic cat lineage were run on a separate gel. Due to the number of individuals tested, two gels were needed for the individuals of the pantherine lineage, with the same five individuals used on both gels to allow band comparisons between gels. For each lineage, the spotted hyena was used as a nonfelid outgroup species, along with at least one felid from outside the lineage represented on the gel. Band sharing and phylogenetic analyses were restricted to species samples compared on a single gel.

Percentage interspecies variation (p) was estimated using Nei and Li's (1979) index of proportion of fragments shared, or of equal size (¶ = pi), computed by FRAG-NEW (developed by J. Avise and M. Ball and modified for larger data sets). This is the equivalent of d_{xy} of the RESTML algorithm of PHYLIP. For maximum-parsimony analysis the presence or absence of fragments were used as characters.

Phylogenetic relationships among individuals within each set of RFLP data were constructed from the distance data by the minimum-evolution method estimated by the neighbor-joining algorithm of the PHYLIP computer package (Version 3.5) (Felsenstein, 1993) and from the character data using the Dollo parsimony model with the heuristic option of PAUP (Version 3.1.1) (Swofford, 1993), followed by the bootstrapping option with 100 resamplings. Dollo parsimony (Farris, 1977) was used because parsimony analysis of restriction-site data may be reliable only when divergences are less than 1% (Nei and Tajima, 1985) and because it is more likely that an existing restriction site will be lost than gained at any particular location (Templeton, 1983; DeBry and Slade, 1985). Dollo parsimony assumes that each character state evolves only once (gains of the derived condition in parallel branches are not permitted) and that all homoplasy is due to a reversal to a more ancestral condition (Swofford, 1993). For comparison, trees were also constructed by maximum parsimony using the heuristic option of PAUP (Version 3.1.1) (Swofford, 1993).

RESULTS

Mitochondrial DNA variation of the three major lineages of Felidae (ocelot, domestic cat, and pantherine lineage) was characterized by RFLP analyses using 28 restriction enzymes. Within the pantherine lineage, 429 restriction sites were scored for 38 individuals of 11 species, representing 2456 nucleotides, or 14.4% of the 17,000 bp in the feline mitochondrial DNA (Lopez *et al.*, 1996). For the domestic cat lineage, 423 restriction sites were scored based on 21 individuals of 12 species representing 2438 nucleotides (14.3% of the mtDNA). With the ocelot lineage, 402 restriction sites were scored for 25 individuals of 13 species, representing 2279 nucleotides (13.4% of the mtDNA).

Twenty-five of 28 restriction enzymes demonstrated intraspecies polymorphism in at least 1 of 17 felid species tested with more than one individual (Tables II and III). Tigrina had multiple banding patterns for the largest number of enzymes (18 of 23), followed by European wild cat (14 of 25) and by margay and Geoffroy's cat (11 of 23). In contrast, no polymorphic sites were detected in the 10 tigers or 4 cheetahs tested.

Table III. The Occurrence of Nuclear Mitochondrial (*Numt*) Fragments in Tested Species[a]

	*Acc*I 4	*Apa*I 3	*Ava*I 3	*Ava*II 7	*Bam*HI 2	*Bcl*I 3	*Bgl*I 0	*Bgl*II 0	*Bst*UI 3	*Bst*EII 2
Lpa	—	—	—	—	—	—	—	—	—	—
Lwi	—	—	—	—	—	—	—	—	—	—
Lti	—	—	—	—	—	—	—	—	—	—
Oge	—	—	—	—	—	—	—	—	—	—
Ogu	—	—	—	—	—	—	—	—	—	—
Fca	2 (7.2)	2 (3.4)	—	—	1 (7.4)	2 (6.7)	1 (8.0)	ND	—	2 (8.0)
Fli	5 (11.5)	2 (3.2)	—	3 (6.2)	1 (7.4)	—	—	ND	—	2 (7.5)
Fsi	2 (7.2)	2 (3.2)	—	2 (5.2)	1 (7.4)	2 (6.7)	—	ND	—	2 (7.5)
Fma	1 (7.5)	2 (7.4)	—	2 (4.2)	1 (7.4)	2 (6.7)	—	ND	—	—
Fch	—	—	—	—	—	—	—	ND	—	—
Fni	—	—	—	—	—	—	—	ND	—	—
Oma	—	—	—	—	—	—	—	ND	—	—
Ppa	ND	—	—	1 (7.6)	2 (6.4)	—	3 (6.0)	ND	—	—
Ple	ND	2 (5.7)	1 (7.8)	1 (8.6)	2 (6.8)	3 (2.2)	1 (3.6)	ND	—	1 (4.7)
Pon	ND	—	1 (14.8)	—	1 (3.6)	—	2 (3.5)	ND	—	1 (4.7)
Pun	ND	2 (9.7)	3 (44.0)	1 (2.2)	—	—	—	ND	—	—
Pti	ND	2 (16.2)	2 (32.6)	2 (6.5)	3 (17.4)	—	2 (3.4)	ND	—	1 (2.7)
Nne	ND	—	—	—	—	—	—	ND	—	—
Hya	—	—	—	—	—	ND	—	—	—	—
Aju	ND	—	—	—	—	—	—	ND	—	—

	*Cla*I 3	*Dra*I 5	*Eco*RI 1	*Eco*RV 1	*Hinc*II 10	*Hin*dIII 4	*Hap*I 6	*Kpn*I 0	*Nco*I 2	*Nde*I 1
Lpa	—	—	—	—	—	—	—	ND	—	—
Lwi	—	—	—	—	—	—	—	ND	—	—
Lti	—	—	—	—	—	—	—	ND	—	—
Oge	—	—	—	—	—	—	—	ND	—	—
Ogu	—	—	—	—	—	—	—	ND	—	—
Fca	—	2 (3.0)	1 (7.6)	1 (8.1)	1 (2.6)	—	—	ND	2 (9.1)	—
Fli	—	2 (3.0)	1 (7.6)	1 (8.1)	2 (6.0)	—	—	ND	3 (11.6)	—
Fsi	—	2 (3.0)	1 (7.6)	1 (8.1)	1 (2.6)	—	—	ND	2 (9.1)	—
Fma	—	1 (1.8)	—	—	1 (2.6)	—	—	ND	1 (1.6)	—
Fch	—	1 (1.8)	1 (7.6)	—	—	—	—	ND	2 (9.1)	—
Fni	—	—	—	—	—	—	—	ND	—	—
Oma	—	—	—	—	—	—	—	ND	—	—
Ppa	—	1 (2.1)	1 (8.6)	—	—	1 (8.1)	1 (3.6)	1 (7.9)	—	ND
Ple	—	1 (2.0)	1 (8.6)	—	—	2 (7.2)	1 (3.6)	1 (7.9)	—	ND
Pon	—	—	—	—	—	—	—	1 (7.9)	—	ND
Pun	1 (5.0)	1 (2.9)	—	—	1 (8.9)	1 (7.3)	1 (8.6)	—	2 (10.8)	ND
Pti	1 (5.0)	6 (12.6)	—	—	1 (2.8)	—	1 (8.6)	—	3 (6.2)	ND
Nne	—	—	—	—	—	—	—	—	—	ND
Hya	—	—	—	—	—	—	—	ND	—	—
Aju	—	—	—	—	—	—	—	—	—	ND

	*Pst*I 0	*Pvu*II 0	*Sal*I 0	*Sst*I 1	*Sst*II 2	*Stu*I 5	*Xba*I 4	*Xho*I 0
Lpa	ND	ND	ND	—	—	—	—	ND
Lwi	ND	ND	ND	—	—	—	—	ND
Lti	ND	ND	ND	—	—	—	—	ND
Oge	ND	ND	ND	—	—	—	—	ND
Ogu	ND	ND	ND	—	—	—	—	ND
Fca	—	—	ND	1 (5.6)	—	1 (2.2)	—	ND
Fli	—	—	ND	1 (5.6)	—	1 (2.2)	—	ND
Fsi	—	—	ND	1 (5.6)	—	1 (2.2)	—	ND

Table III. Continued

	*Pst*I 0	*Pvu*II 0	*Sal*I 0	*Sst*I 1	*Sst*II 2	*Stu*I 5	*Xba*I 4	*Xho*I 0
Fma	—	—	ND	1 (5.6)	—	—	—	ND
Fch	—	—	ND	1 (5.6)	—	—	—	ND
Fni	—	—	ND	—	—	—	—	ND
Oma	—	—	ND	—	—	—	—	ND
Ppa	1 (4.5)	1 (8.3)	—	1 (2.3)	—	ND	1 (4.7)	—
Ple	2 (8.4)	1 (8.3)	1 (8.4)	2 (6.6)	—	ND	4 (8.4)	—
Pon	1 (4.5)	—	—	2 (6.6)	—	ND	—	—
Pun	2 (12.8)	—	—	4 (16.9)	—	ND	1 (4.7)	—
Pti	1 (9.3)	1 (12.0)	—	4 (11.2)	—	ND	3 (16.0)	—
Nne	—	—	—	—	—	ND	—	—
Hya	—	ND	ND	—	—	—	—	ND
Aju	—	—	—	—	—	—	—	—

[a] Below the restriction enzyme is the number of restriction sites in the domestic cat *Numt* sequence (Lopez *et al.*, 1994). The number of fragments in each species (codes from Table II) is followed, in parentheses, by the total kb size of the *Numt* fragment(s). (—) No *Numt* sequences were observed in this species' restriction enzyme combination. ND refers to enzymes which were not tested with this species.

Mitochondrial DNA evolution in the Felidae is somewhat complicated by the occurrence of *Numt*, an ancient tandem amplification of 7.9 kb of mtDNA located on nuclear chromosome D2 in domestic cats and also in closely related species (Lopez *et al.*, 1994). Because of the high copy number (38–56*x*) of *Numt* present in nuclear DNA, Southern blot fragments in species with *Numt* consist of both cytoplasmic mtDNA and nuclear *Numt* fragments. To discriminate between *Numt* and cytoplasmic mtDNA in the present study, purified cytoplasmic (nonnuclear) DNA preparations were compared to whole-cell DNA preparations to identify species with *Numt* fragments (Fig. 1). Ten species displayed *Numt* specific nuclear RFLP signals (Fig. 1 and Table III), five from the domestic cat lineage (*F. catus*, *F. silvestris*, *F. libyca*, *F. margarita*, and *F. chaus*) and five from species of the genus *Panthera* (*P. tigris*, *P. leo*, *P. pardus*, *P. onca*, and *P. uncia*). Because these species represent monophyletic lineages within the felid radiation, it is likely that *Numt* amplification across two disparate lineages represents two unique evolutionary events. *Numt* fragments were not considered for construction of phylogenetic relationships.

Pairwise percentage interspecies variation (p) (Nei and Li, 1979) varied from 1 to 19% among all cats, including outgroup species (Tables IV–VI). Interspecies variation reached 16% within the pantherine lineage (between leopard and snow leopard), 12% within the ocelot lineage (between some individuals of Geoffroy's cat and margay), and 7% within the domestic cat lineage (between Pallas cat and black-footed cat).

Ocelot Lineage

Phylogenetic analysis of mtDNA from species of the ocelot lineage revealed three groups which were well supported by distance based and Dollo parsimony methods (Figs. 2A and B). These groups, composed of Geoffroy's cat (Oge) and kodkod (Ogu), ocelot (Lpa) and margay (Lwi), and pampas cat (Lco) and three of the tigrinas (Lti), had boot-

Table IV. Number of Different Restriction Sites Out of 402 (Top) and Percentage Interspecies Variation (Index of Proportion of Mitochondrial Restriction Length Fragments Not Shared; *p*) for Species of the Ocelot Lineage (Bottom)

	Lpa3	Lpa6	Lpa7	Lwi1	Lwi8	Lwi12	Lti3	Lti5	Lti65	Lco4	Lti8	Oge3	Oge8	Oge11	Oge66	Ogu2	Ogu3	Hya	Aju	Ccr2
Lpa3	—	8	17	70	64	68	79	76	73	71	84	89	90	93	96	84	88	95	98	94
Lpa6	0.38	—	17	66	64	68	79	76	73	69	82	96	90	93	96	82	86	91	96	90
Lpa7	0.90	0.99	—	59	57	61	74	71	72	68	81	89	85	88	89	83	87	92	95	89
Lwi1	5.31	5.03	4.16	—	24	24	77	74	73	69	80	89	90	91	94	88	92	85	90	92
Lwi8	4.62	4.72	3.85	1.32	—	4	5	79	71	67	82	83	84	85	88	82	86	89	94	100
Lwi12	5.04	5.14	4.26	1.40	0.22	—	85	82	75	71	86	85	86	87	90	84	88	91	96	100
Lti3	6.00	6.09	5.15	5.52	5.78	6.18	—	5	20	32	65	92	93	92	95	89	93	88	105	101
Lti5	5.89	5.97	5.04	5.38	5.64	6.04	0.21	—	15	35	68	89	90	89	92	86	90	87	102	98
Lti65	5.70	5.85	5.52	5.55	5.13	5.52	1.04	0.81	—	18	69	90	91	92	95	87	91	88	97	97
Lco4	5.52	5.44	4.95	5.39	4.94	5.37	1.81	1.67	1.03	—	65	88	89	90	93	83	87	84	95	91
Lti8	11.17	11.17	6.31	10.40	9.97	11.12	4.90	5.16	10.37	4.69	—	85	88	91	96	84	88	91	104	106
Oge3	7.13	7.53	6.13	11.67	10.27	11.22	6.90	6.77	7.51	7.73	6.26	—	7	12	17	23	25	96	105	101
Oge8	7.35	7.75	6.33	11.93	10.51	11.46	7.11	6.97	7.74	7.94	6.60	0.34	—	15	20	28	30	95	104	100
Oge11	7.30	7.68	6.46	7.41	6.47	6.70	6.69	6.55	7.37	7.63	6.79	0.56	0.65	—	5	31	33	98	107	101
Oge66	7.64	8.02	6.51	7.74	6.78	7.00	6.97	6.83	7.69	7.92	7.26	0.77	0.88	0.19	—	36	32	103	110	102
Ogu2	6.56	6.48	6.15	7.06	6.10	6.29	6.81	6.65	7.33	6.61	5.82	1.80	1.46	1.54	1.80	—	4	93	104	96
Ogu3	6.89	6.81	6.51	7.45	6.47	6.65	7.10	6.94	7.63	6.94	6.12	1.56	1.54	1.62	1.56	0.16	—	97	108	96
Hya	10.15	9.70	9.46	8.80	9.06	9.23	7.67	7.95	8.77	8.58	7.97	11.02	9.33	9.59	11.02	8.85	9.54	—	87	81
Aju	10.18	10.07	9.25	8.62	9.29	9.43	10.64	10.48	10.63	9.37	14.49	20.67	24.29	20.26	20.67	20.69	21.07	9.37	—	98
Ccr2	10.11	9.63	8.38	9.57	11.42	11.13	10.20	10.06	11.16	9.69	15.48	10.46	15.12	10.42	10.46	9.08	8.78	11.16	9.69	—

Table V. Number of Different Restriction Sites Out of 423 (Top) and Percentage Interspecies Variation (Index of Proportion of Mitochondrial Restriction Length Fragments Not Shared; *p*) for Species of the Domestic Cat Lineage (Bottom)

	Fca84	Fli2	Fsi7	Fsi10	Fsi12	Fma	Fni3	Fni6	Fni7	Fch	Oma	Aju	Ple	Ccr
Fca84	—	18	11	28	17	44	90	94	90	81	100	86	116	164
Fli2	0.67	—	17	34	20	44	94	98	94	85	96	116	128	130
Fsi7	0.39	0.63	—	21	15	43	89	93	89	82	101	115	123	139
Fsi10	1.05	1.35	0.77	—	24	52	96	100	96	89	104	122	117	138
Fsi12	0.66	0.76	0.55	0.88	—	85	94	98	94	85	100	117	124	138
Fma[a]	1.86	1.91	1.81	2.22	1.65	—	86	88	86	97	102	122	118	164
Fni3	4.39	4.92	4.30	4.80	4.40	4.21	—	6	4	97	112	120	134	142
Fni6	4.95	5.49	4.73	5.39	5.00	4.41	0.26	—	7	97	110	120	134	142
Fni7	4.39	4.92	4.30	4.80	4.40	4.21	0.12	0.33	—	97	110	120	134	142
Fch[b]	3.99	4.43	4.00	4.55	4.14	5.53	4.96	5.15	4.96	—	99	125	123	135
Oma[c]	6.22	6.07	6.29	6.55	6.25	7.01	7.42	7.28	7.21	6.07	—	120	128	114
Aju	8.37	9.00	8.44	9.20	8.89	10.72	8.13	8.67	8.13	9.41	8.49	—	128	128
Ple	11.18	10.58	10.78	11.77	11.19	12.90	11.99	12.51	11.99	10.58	12.07	11.14	—	128
Ccr	12.64	11.80	13.43	12.72	13.38	13.26	12.35	12.96	12.35	12.55	9.40	12.80	15.23	—

[a] Includes Fma5, Fma10, and Fma11.
[b] Includes Fch2 and Fch4.
[c] Includes Oma4 and Oma10.

Table VI. Number of Different Restriction Sites Out of 429 (Top) and Percentage Interspecies Variation (Index of Proportion of Mitochondrial Restriction Length Fragments Not Shared; *p*) for Species of the Pantherine Lineage (Bottom)

	Ppa106	Ppa21	Ppa9	Ppa30	Ple23	Ple	Ple24	Pon1	Pon9	Pun	Pun10	Pti	Nne	Aju	Ccr
Ppa106	—	5	5	12	92	91	89	85	87	93	92	31	88	93	101
Ppa21	0.12	—	1	5	71	61	57	85	88	90	89	30	87	72	100
Ppa9	0.12	0.00	—	7	93	88	90	84	87	90	90	28	85	90	100
Ppa30	0.49	0.38	0.38	—	92	85	89	83	86	95	94	23	84	89	101
Ple23	10.63	6.59	6.59	6.73	—	65	68	81	85	83	80	89	74	79	101
Ple[a]	10.43	6.37	6.37	6.50	0.25	—	5	82	86	88	85	84	81	84	106
Ple24	10.43	6.37	6.37	6.50	0.25	0.07	—	78	82	86	85	86	71	76	98
Pon1	12.80	12.80	12.80	12.78	12.69	12.68	12.68	—	4	86	91	82	57	64	100
Pon[b]	13.19	13.19	13.19	13.18	12.68	12.67	12.67	0.53	—	88	90	84	61	68	100
Pun[c]	15.66	15.66	15.66	16.23	11.75	11.42	11.42	11.37	11.55	—	4	25	87	88	96
Pun10	15.62	15.62	15.62	16.19	11.48	11.15	11.15	11.63	11.61	0.24	—	27	72	81	81
Pti[d]	7.17	7.17	7.17	7.09	10.05	9.82	9.82	12.46	12.17	5.79	5.90	—	83	84	96
Nne[e]	13.30	12.86	12.86	13.21	12.42	12.06	11.74	10.29	10.25	10.49	10.58	14.29	—	17	89
Aju[f]	18.81	18.81	18.81	18.46	9.97	9.09	9.09	18.55	18.94	17.80	15.29	16.55	11.58	—	94
Ccr2	22.46	22.46	22.46	24.45	21.28	20.63	20.63	11.89	11.85	14.85	14.62	15.85	14.69	11.34	—

[a] Includes Ple7, Ple13, Ple23, Ple24, and Ple105.
[b] Includes Pon1, Pon9.
[c] Includes Pun4, and Pun9.
[d] Includes Pti2, Pti48, Pti65, Pti66, Pti69, Pti76, Pti77, Pti81, Pti82, and Pti83.
[e] Includes Nne9, Nne19, and Nne22.
[f] Includes Aju70, Aju96, Aju227, and Aju254.

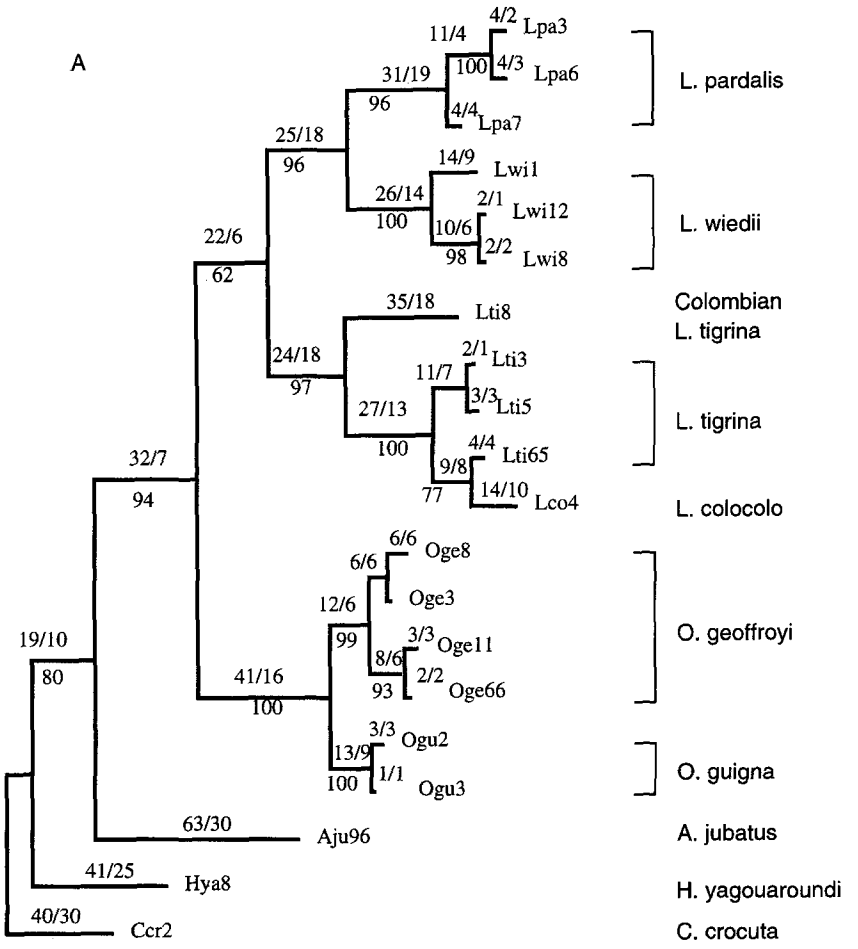


Fig. 2. MtDNA RFLP phylogenetic trees for species of the ocelot lineage. (A) Tree derived from Dollo parsimony algorithm (N = 402 character states, tree length = 575 steps, CI = 0.515). Numbers above the branches correspond to the number of steps/number of homoplasies, and numbers below to bootstrap percentages from 100 iterations. (B) Tree derived from minimum-evolution method estimated by neighbor-joining algorithm (numbers refer to branch lengths). Time scale represents estimated times of divergence based on fossil calibrations (see text).

strap values ranging from 96 to 100%. The depth of the nodes uniting the two species of each clade differed, suggesting that the ocelot and margay diverged prior to the other two clades. The four tigrina samples separated into two distinct groups: those of Brazilian origin (Lti3, -5, -65) aligned with pampas cat, and an individual of Colombian origin (Lti8) formed an outgroup of the tigrina/pampas cat clade. Genetic divergence (p = 4.9–5.2%) between these two groups of tigrinas was five times greater than among Brazilian tigrina samples (p = 0.2–1.0%) and comparable to the genetic distance observed between species (Table IV). Depending upon the method used, the relationships among the three groups of ocelot lineage species varied, placing either the ocelot/

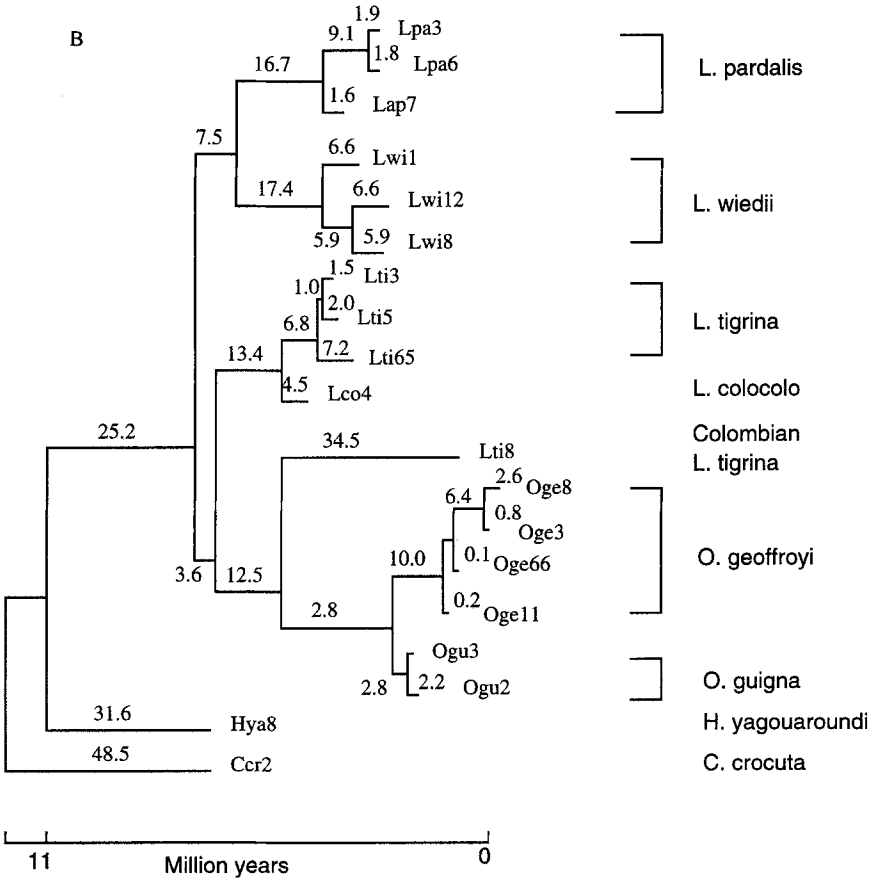


Fig. 2. Continued.

margay clade (Fig. 2B) or the Geoffroy's cat/kodkod clade (Fig. 2A) as more plesiomorphic. The occurrence of 22 fragment steps (16 synapomorphic, 6 homoplastic) aligning the ocelot/margay with the tigrina/pampas cat provides modest parsimony support (bootstrap = 62%) favoring an earlier divergence of a Geoffroy's cat/kodkod ancestor.

Mean nucleotide diversity between species of the ocelot lineage and the cheetah (Aju) of the pantherine lineage was 14.12% (Table IV). Based on the fossil record, the ocelot lineage last shared a common ancestor with cheetah 10–12 MYA (Werdelin, 1985; Wayne *et al.*, 1989). If we assume a constant rate of mitochondrial RFLP change, the rate would be 1.18–1.41%/MY. Ocelot and margay therefore would have diverged 3.3–4.0 MYA and the most recent common ancestor among these South American small cats would have occurred 5–6 MYA (Fig. 2B).

Domestic Cat Lineage

Dollo parsimony and distance-based analysis of domestic cat species produced similar topologies (Fig. 3). Parsimony analysis resulted in three minimum-length trees

(length = 606 steps; CI = 0.584) which differed only in the relationships among the domestic cat and the European and African wild cats. Domestic cat (Fca), European wild cat (Fsi), and African wild cat (Fli) form an unresolved trichotomy, which joined with the sand cat (Fma) by a common ancestor (92% of bootstraps), and represent the most recent radiations within the domestic cat lineage. Jungle cat (Fch) and black-footed cat (Fni) diverged earlier and are less closely related (p = 5%, compared with approximately 1% among domestic cat, African wild cat, and European wild cat) (Table V). Jungle cat appeared to diverge prior to black-footed cat in the distance-based analysis but became an unresolved trichotomy in the parsimony analysis with black-footed cat and the sand cat/domestic cat clade. Placement of Pallas cat (Oma) within the domestic cat lineage is weakly supported by maximum-parsimony analysis, which placed Pallas cat within the clade of domestic cats in 70% of the bootstraps when cheetah and spotted hyena were outgroup species.

The mean variation between species of the domestic cat lineage and the cheetah was 9.00% (Table V). Assuming that they shared a common ancestor around 8–10 MYA (Wayne *et al.*, 1989), a constant rate of mitochondrial RFLP change is 0.9–1.12%/MY. Domestic cats/African and European wild cats are estimated to have diverged from sand cats approximately 1.7–2.1 MYA (Fig. 3B).

Pantherine Lineage

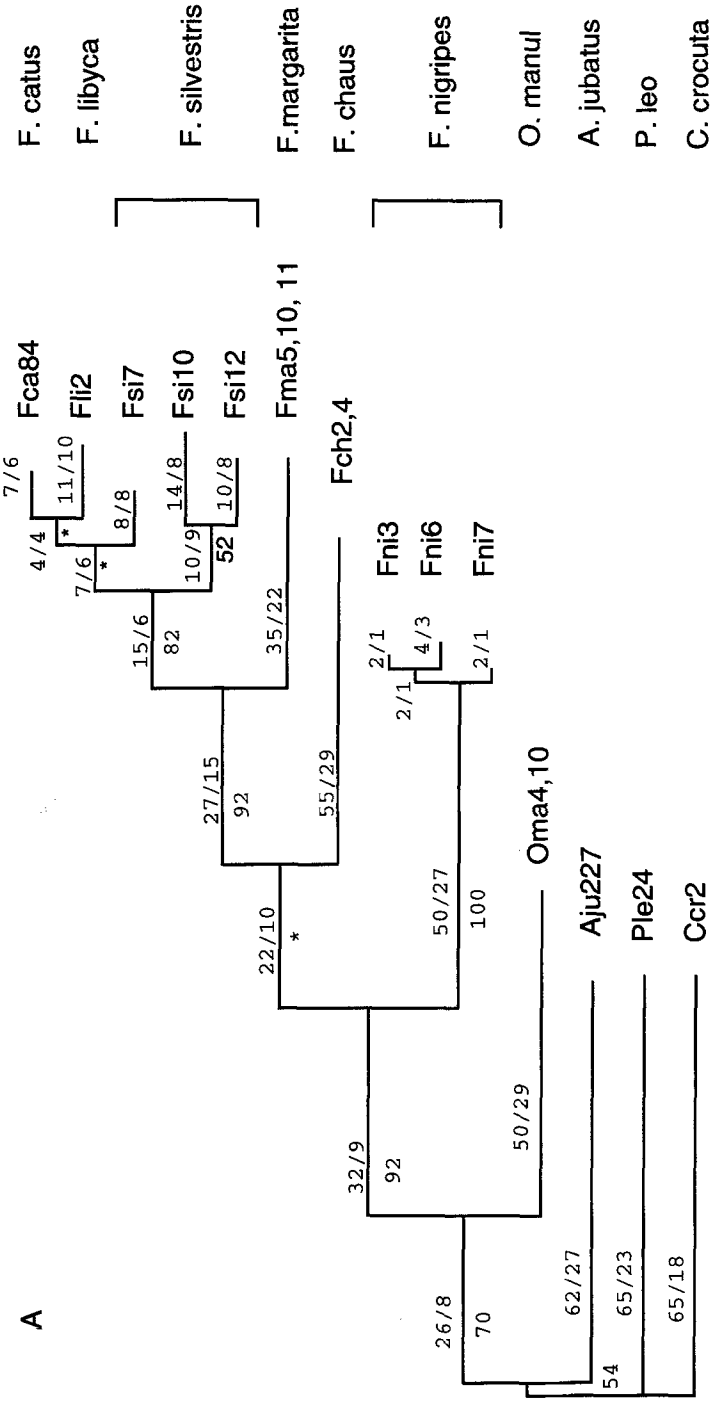
Clouded leopard (Nne) was the most primitive offshoot of the six species in this group. Dollo parsimony analysis produced two equally parsimonious minimum-length trees of length 623 steps (CI = 0.587) (Fig. 4A). These trees supported the association of tiger (Pti) and snow leopard (Pun) (bootstrap proportion = 70%), but the relationship of leopard (Ppa) and lion (Ple) was more weakly supported (bootstrap proportion = 55%). Minimum-evolution analysis using the neighbor-joining algorithm also implicated the lion/leopard and tiger/snow leopard as monophyletic groups (Fig. 4B).

Mean variation among species of the *Panthera* genus (including clouded leopard) and the cheetah was 15.32% (Table VI). Assuming that they shared a common ancestor with cheetah 3.5–4.5 MYA (Ficcarelli, 1984; Turner, 1987), their rate of mitochondrial RFLP change was 3.40–4.37%/MY. Lions and leopards therefore would have shared a common ancestor 2.0 MYA, while the date of the *Panthera* common ancestor is approximately 3.0 MYA (Fig. 4).

DISCUSSION

Phylogenetic analysis of mtDNA variation within the three major clades of Felidae corroborated their different patterns of evolutionary history. Members of the ocelot lineage are distantly related to the other felid species but diverged in a relatively short time into several lineages. In contrast, species of the domestic cat lineage separated from the other felids more recently and, with the exception of the domestic cat/European and African wild cat group, are separated from each other by longer branch lengths. Members of the *Panthera* genus are closely interrelated, suggestive of recent and rapid speciation.

Within the ocelot lineage, our results concur with the results of two-dimensional


A
F. catus
F. libyca
F. silvestris
F.margarita
F. chaus
F. nigripes
O. manul
A. jubatus
P. leo
C. crocuta
Fca84
Fli2
Fsi7
Fsi10
Fsi12
Fma5,10, 11
Fch2,4
Fni3
Fni6
Fni7
Oma4,10
Aju227
Ple24
Ccr2
7/6
4/4
11/10
*
7/6
*
8/8
14/8
10/9
52
10/8
15/6
82
35/22
27/15
92
55/29
2/1
4/3
2/1
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26/8
70
62/27
54
65/23
65/18

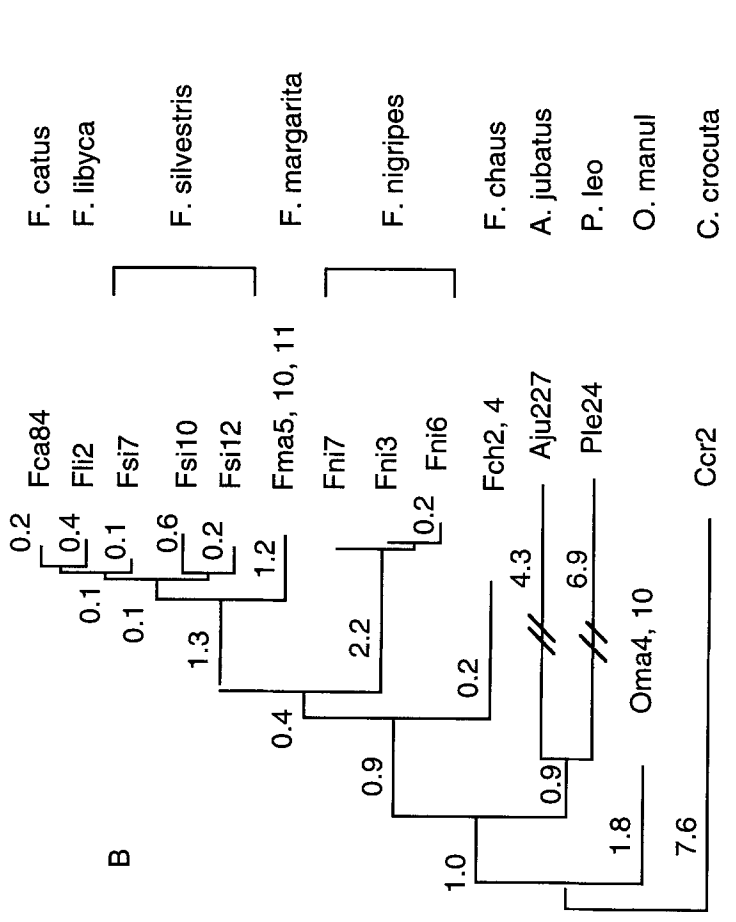
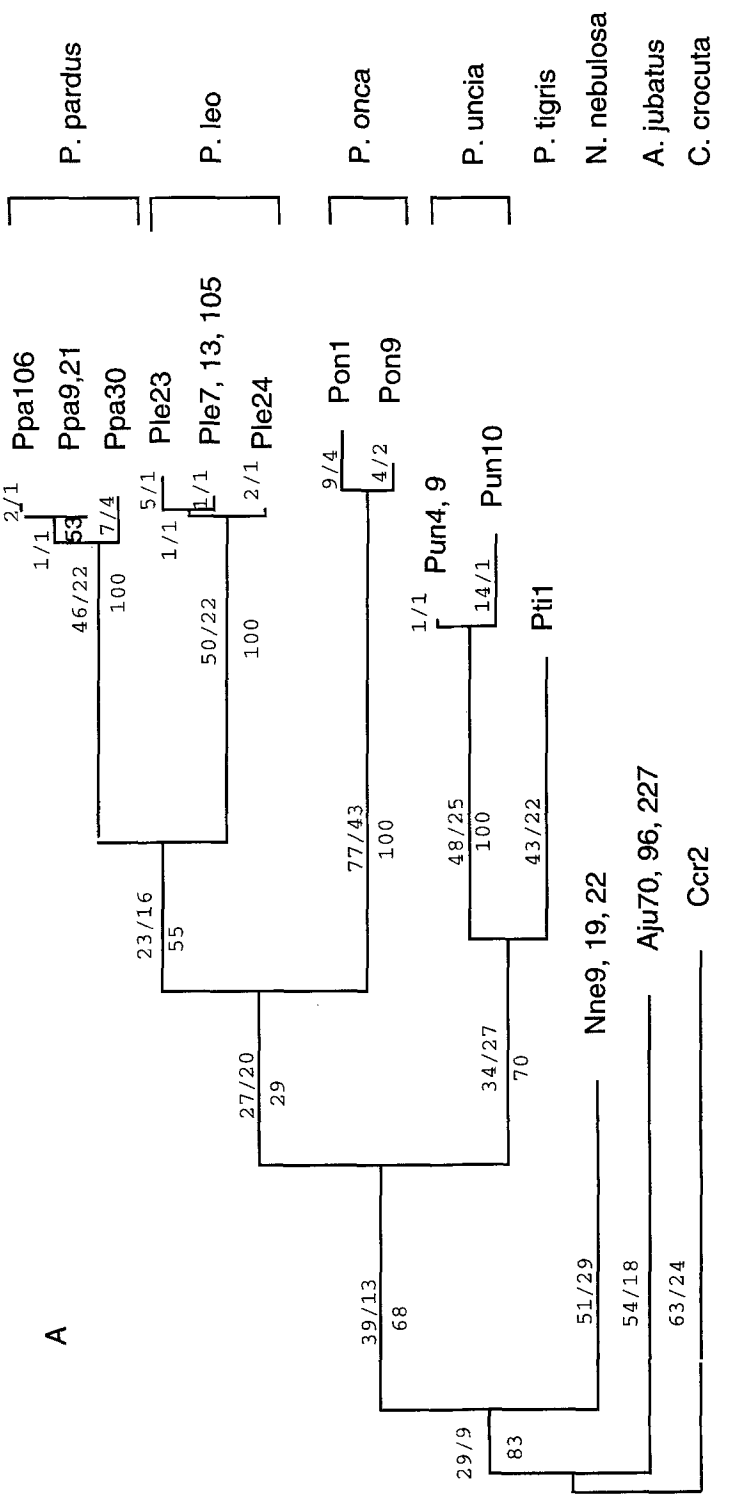


Fig. 3. MtDNA RFLP phylogenetic trees for species of the domestic cat lineage. (A) Tree derived from Dollo parsimony algorithm (three most parsimonious trees; $N = 423$ character states, tree length = 606 steps, CI = 0.584). Numbers above the branches correspond to the number of steps/number of homoplasies, and numbers below to bootstrap percentages from 100 iterations. Asterisks represent nodes where the bootstrap tree differed from the parsimony tree. (B) Tree derived from minimum-evolution method estimated by neighbor-joining method (numbers refer to branch lengths). Time scale represents estimated times of divergence based on fossil calibrations (see text).


A
P. pardus
P. leo
P. onca
P. uncia
P. tigris
N. nebulosa
A. jubatus
C. crocuta
Ppa106
Ppa9,21
Ppa30
Ple23
Ple7, 13, 105
Ple24
Pon1
Pon9
Pun4, 9
Pun10
Pti1
Nne9, 19, 22
Aju70, 96, 227
Ccr2
2/1
1/1
53
7/4
46/22
100
5/1
1/1
1/1
50/22
100
2/1
23/16
55
9/4
4/2
77/43
100
1/1
14/1
48/25
100
43/22
27/20
29
34/27
70
39/13
68
51/29
54/18
63/24
29/9
83

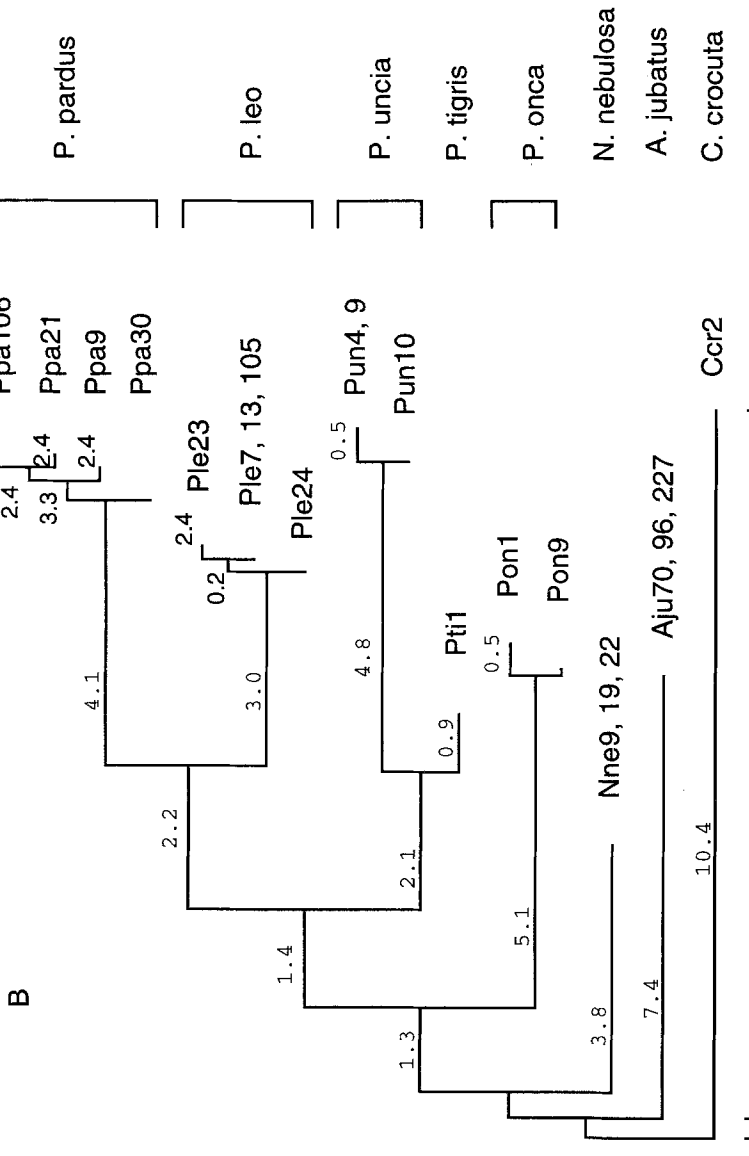


Fig. 4. MtDNA RFLP phylogenetic trees for species of the pantherine lineage. (A) Tree derived from Dollo parsimony algorithm (four most parsimonious trees; N = 429 character states, tree length = 623 steps, CI = 0.587). Numbers above the branches correspond to the number of steps/number of homoplasies, and numbers below to bootstrap percentages from 100 iterations. (B) Tree derived from minimum-evolution method estimated by neighbor-joining method (numbers refer to branch lengths). Time scale represents estimated times of divergence based on fossil calibrations (see text).

protein electrophoresis and allozyme analyses, which combine ocelot and margay into a separate clade and affiliate Geoffroy's cat with kodkod (Pecon Slattery *et al.*, 1994). However, the relationships among tigrina, pampas cat, and the other four species differ between the protein electrophoretic methods and the RFLP analysis. This difference in topology may be the result of sampling because the present data suggest that there may be two divergent phylogenetic clades of tigrina, one made up of individuals from Brazil and the other, more divergent clade, composed of individuals from Colombia. Colombian tigrina samples were not available for the protein analyses (Pecon Slattery *et al.*, 1994).

Morphological evidence provides limited support for these results. The close relationship between ocelot and margay has invariably been supported by morphological studies (Glass and Martin, 1978; Salles, 1992). Further, ocelot and margay also share a unique deletion of chromosome D2 (Modi and O'Brien, 1988). There has been little agreement, however, as to the relationships among the other species of the lineage (Hemmer, 1978; Herrington, 1986; Salles, 1992).

Divergence dates within the ocelot lineage estimated from RFLP data are consistent with those calculated from the two-dimensional electrophoretic data. Both estimates suggest that ocelot species diverged initially around 6 MYA, and differentiated further 2–5 MYA. The timing of this radiation coincides with the formation of the land bridge between North and South America, before which there were no eutherian mammal carnivores (including cats) in South America (Martin, 1989; Wayne *et al.*, 1991). This chronology is also fairly consistent with the fossil record. The earliest predecessors of the ocelots (*F. lacustris* or *F. rexrodenosis*) appeared 4–5 MYA. Fossils of modern *Leopardus* species date to approximately 1.5–2.5 MYA in North America and 0.3–0.4 MYA in South America (Berta, 1983).

The evolutionary history of the domestic cat lineage produces a topology which is described fairly consistently by the genetic methods employed to date (Collier and O'Brien, 1985; Modi and O'Brien, 1988; Lopez *et al.*, 1994). Both 12S rRNA sequences (Lopez *et al.*, 1994) and mtDNA RFLP determine that the most distantly related species of the lineage is the Pallas cat (about 6 MYA), which also differs from the other species by a chromosomal inversion (Wurster-Hill and Centerwall, 1982) and the absence of endogenous RD114 retroviral sequences (Benveniste, 1985). The second group to emerge is the jungle cat and the black-footed cat, which diverged prior to other *Felis* species, although the available data conflict as to whether the jungle cat or black-footed cat diverged first. The mtDNA RFLP data give conflicting results depending on the method of analysis (Fig. 3). Concurrent studies examining mtDNA sequence data have also been unable to resolve these interrelationships. Sequences of mtDNA 12S rRNA favor the jungle cat as diverging first, while cytochrome *b* sequences favor the black-footed cat as being earlier (Masuda *et al.*, 1996). Perhaps a stronger character to consider would be the presence of *Numt* in the jungle cat but not in the black-footed cat. If this remarkable nuclear transposition and amplification of the mtDNA genome were considered as a principal and unique evolutionary event, it would suggest that the black-footed cat lineage was likely a more primitive divergence than the other *Felis* species.

MtDNA RFLP data appear to resolve the most recent domestic cat radiation, placing the sand cat as a plesiomorphous outgroup of domestic and European and African wild cats (1.6 MYA). The earliest fossil record from this lineage, of *Felis lunensis*

(3 MYA), does not contradict our findings, but fossil evidence for this lineage is generally sparse (Kurtén, 1965). There has been little consistency among the results of different morphological studies on the domestic cat lineage, except often to align closely European wild cat and African wild cat (Randi and Ragni, 1991) and to distinguish Pallas cat from the rest of the lineage (Hemmer, 1978; Herrington, 1986; Salles, 1992). The composite results of morphology, cytology, 12S rRNA sequences, cytochrome *b* sequences, and mtDNA RFLP analyses affirm the suggestion that European wild cat and African wild cat be subsumed under the same species name, *Felis silvestris*, in agreement with Wozencraft (1993).

Relationships among species of the *Panthera* genus, which evolved recently and rapidly, are difficult to resolve with the genetic methods applied thus far (O'Brien *et al.*, 1987). This may be due in part to transmission of substantive ancestral genetic polymorphisms (Wu, 1991) common to recent monophyletic radiations that retain ancestral genetic variation. Within the *Panthera* genus the mtDNA RFLP data indicate a recent relationship between tiger and snow leopard. This finding differs from the results of Janczewski *et al.* (1995), who found no support for aligning tiger with snow leopard from analysis of cytochrome *b* and 12S rRNA sequences but, instead, suggested that tiger and clouded leopard may be sister taxa. However, their results, especially with 12S rRNA, suggested that there was a common ancestry between lion and leopard (2.0 MYA), a result weakly implied by the restriction-site analysis (Fig. 4). A close relationship between lion and leopard also has been suggested by several morphological studies (Hemmer, 1978; Herrington, 1986; Salles, 1992), as has the affinity between tiger and snow leopard (Hemmer, 1978; Herrington, 1986).

The timing of divergence of the nodes within the *Panthera* genus is consistent with fossil evidence. The earliest lion fossils date between 1 and 2 MY (Neff, 1982) and leopard fossils date 3.0 MY from the mid-Pliocene (Savage and Russell, 1983; Turner, 1987), within the range of the suggested divergence between the two species of 2.0 MY. The existence of 1.8-MY-old tiger-like fossils (Neff, 1982) suggests, however, that the divergence of tigers may have been somewhat earlier than our 1.5-MY estimate between snow leopard and tiger.

Rates of mtDNA Divergence

The rates of base-pair change within the lineages, calculated from when each lineage was estimated last to share a common ancestor with the cheetah, ranged from 1.0%/MY for the domestic cat lineage to 3.8%/MY for the *Panthera* genus. These are within the range of values commonly cited for mitochondrial evolution (Brown, 1985; Martin *et al.*, 1992; Martin and Palumbi, 1993). Although these values represent a relatively large amount of variation within the same family, they should be interpreted with caution. Although significant mtDNA rate heterogeneities have been shown across lineages of Hawaiian *Drosophila* (DeSalle and Templeton, 1988) and turtles (Avise *et al.*, 1992), the cause of these differences is unclear. One possibility is that the mtDNA haplotype date could be later than the actual ancestral population if mtDNA diversity has been lost during evolution (Wolpoff, 1989). The probability that diversity is lost is higher in small, nonexpanding populations (Avise *et al.*, 1984).

These rate estimates for Felidae should also be used cautiously since calibrations

from the felid fossil record are poor and because mtDNA variation tends to decelerate after 8% divergence (Moritz *et al.*, 1987). This deceleration in divergence rate is apparent when comparing the index of proportion of shared mitochondrial restriction fragments of spotted hyena (Ccr) and cheetah with other felid species (Tables IV–VI). Although spotted hyena last shared a common ancestor with felids about 38.5 MYA (Hunt, 1989), the mean divergence between hyena and Felidae species was similar to those among more distantly related cat species (with divergences less than 12 MYA). For example, the mean divergence between hyena and ocelot lineage species was 10.7% (compared with 14.2% between cheetah and ocelot lineage species), 12.1% between hyena and domestic cat lineage species (compared with 9.0%), and 16.8% between hyena and pantherine lineage species (compared with 14.2%).

The phylogenetic relationships and genetic patterns discerned from mtDNA RFLP should be confirmed with other mitochondrial and nuclear genes, because relationships determined from mtDNA data may not always conform with true species phylogenies, particularly within recent mammal radiations such as occurred among Felidae species (Cronin, 1991). The apparent large differences in interspecific variation among felid species also merit further study to determine their cause.

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