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MITOCHONDRIAL DNA VARIATION AND GEOGRAPHIC POPULATION STRUCTURE IN THE YELLOW MONGOOSE (*CYNICTIS PENICILLATA*)

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by

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DEGREE: MSc (Scientiae) Zoology

ABSTRACT

Geographic population structure was determined for *Cynictis penicillata*, a carnivore species endemic to southern Africa. Restriction fragment length polymorphisms (RFLPs) were analyzed for 50 specimens from 21 localities. The 18 restriction endonucleases used in this analysis revealed 13 haplotypes. Low sequence divergence values separate most of these maternal lineages. With the exception of two haplotypes (E and F) which are distributed over a broad geographic range, most lineages are geographically localized. It is postulated that this is the result of a recent population bottleneck followed by a rapid range expansion.

Polymerase Chain Reaction (PCR) amplification of a 600 bp fragment from the 5' end of the *Cynictis* mitochondrial DNA control region revealed the presence of a length polymorphism. Direct nucleotide sequencing of five yellow mongoose specimens revealed the length polymorphism to be due to a direct repeat of 81 bp in the larger fragment.

for Mom

Hanlie Jansen van Vuuren (1932 - 1995)

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CHAPTER 1
INTRODUCTION

HISTORIC OVERVIEW OF THE ORDER CARNIVORA

Origin

Mammals arose from therapsid reptile ancestors in the early Mesozoic (200-135 myr ago). The oldest fossils, unequivocally referable to the class Mammalia, have been recorded from European deposits dating back to the late Triassic (DeBlase & Martin 1982); these progenitors of modern mammals were thought to be either insectivorous or omnivorous (Martin 1989) and it was only in the late Palaeocene and early Eocene (approximately 70-50 myr ago) that true representatives of the order Carnivora evolved (Carroll 1988).

The first fossil evidence of modern mongooses was discovered in France and dates back to the upper Oligocene. Strikingly, although creodont representatives have been found in Africa, the paleontological record is noteworthy for the absence of true carnivore material. In fact, amphicyonids, viverrids, herpestids, felids and nimravids only became abundant in Africa in the middle Miocene (Martin 1989). The absence of American fossil representatives of the otherwise abundant herpestids and viverrids is suggestive that these species evolved at low latitudes since the only connection between Eurasia and North-America (from the late Eocene onwards) was the Beringian route. This high latitude land-bridge made crossing for species that evolved at low latitudes difficult (Martin 1989). In South Africa, the earliest evidence of the yellow mongoose and its ancestral forms are found in fossil deposits of the Makapanian mammal age (approximately 3 myr to 700 000 years ago) at the Makapansgat and Swartkrans sites (Hendey 1974).

Familial relationships

The order Carnivora comprises a heterogenous complex of species with diverse life histories. The plethora of adaptations in carnivores has led to several instances of parallel and convergent evolution of morphological traits (Wayne, Benveniste, Janczewski & O'Brien 1989; Wozencraft 1989a) which has made it extremely difficult for systematists to determine relationships between taxa. The tracing of evolutionary lineages among extinct and living representatives of the order has relied on the diagnostic pattern of ontogenetic elements forming the auditory bulla enclosing the middle ear, as well as the carotid circulation to the brain (Hunt 1987; Carroll 1988). On the basis of these diagnostic patterns modern carnivores are divided into two super families: the Aeluroidea, which includes the viverrids, felids and hyaenids and the Arctoidea, encompassing the canids, procyonids, ursids, mustelids and marine carnivores (Meester, Rautenbach, Dippenaar & Baker 1986; Carroll 1988; Martin 1989; Wayne *et al.* 1989).

The taxonomic placement of the civets, genets and mongooses within the superfamily Aeluroidea has resulted in two main schools of thought. The first argues subfamily status for both the mongooses (Herpestinae) and the civets and genets (Viverrinae) within the family Viverridae (Michaelis 1972; Rosevear 1974; Meester *et al.* 1986). The second, while recognizing the division of the family Viverridae into two groups, finds justification for two separate families, the family Viverridae which includes the civets and genets, and the family Herpestidae which is further divided into the subfamilies Galiidinae, Herpestinae and Mungotinae (Fredga 1972; Radinsky 1975; Bugge 1978; Honacki, Kinman & Koeppl 1982; Hunt 1987; Wozencraft 1989b). Under this scheme the yellow mongoose, *Cynictis penicillata*, falls within the family Herpestidae, subfamily Mungotinae.

SYSTEMATICS OF THE YELLOW MONGOOSE *Cynictis penicillata* (Cuvier, 1829)

The taxonomic status of *C. penicillata* is enigmatic. The yellow mongoose was first described by Cuvier (1829) as belonging to the genus *Herpestes*. Four years later Ogilby (1833) proposed the genus *Cynictis*, and the yellow mongoose was reclassified. When the term subspecies came into general usage during the nineteenth century, it replaced the term variety in its meaning of geographic race. It was considered a taxonomic unit like the morphological species, but at a lower taxonomic level. Many early authors used the term subspecies indiscriminately for describing entities that were less distinct than species (Mayr & Ashlock 1991). This is manifested in the yellow mongoose where 12 subspecies were recognized based on coloration and other morphological characters (Shortridge 1934; Allen 1939; Roberts 1951; Ellerman, Morrison-Scott & Hayman 1953).

In practice, the subspecies category was useful for emphasizing that populations can be different, yet belong to the same species, and were thus believed to reflect adaptations to the local environment and to represent incipient species (Mayr & Ashlock 1991). However, a shift in attitude occurred so that where variation was found to be clinal in continuously distributed populations, it was clear that no particular benefit accrues from splitting the continuum into several subspecies. As a result of this, many subspecies that were named from the 1920's to the 1950's have been placed in synonymy. Lundholm (1955) concluded that previous authors based their description of the yellow mongoose subspecies on seasonal variation, and proposed the term cline-complex to describe the variation within *Cynictis*. Cline-complex, and not cline, was chosen since the different characters reach their extremes within different local forms and the variation is not linear. This prompted Meester and co-workers (1986) to view *C. penicillata* as monotypic, a trend which was subsequently followed by Skinner and Smithers (1990).

Most recently, Taylor (1990) recognized the phenetic delimitation of four parapatric subspecies for *C. penicillata* based mainly on skull morphology (Fig.1): *C. p. penicillata*

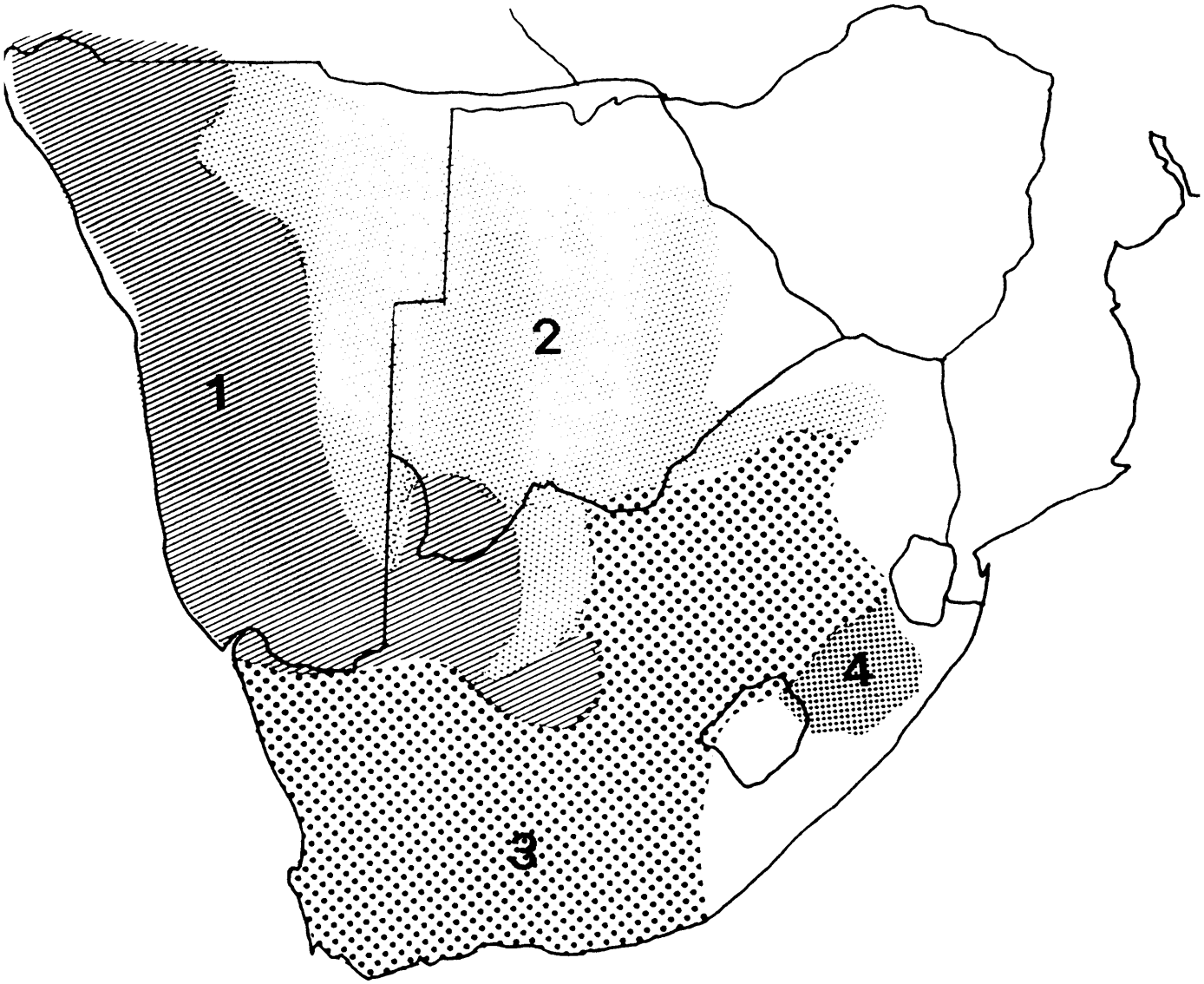

Figure 1: Morphometric delimitation of four parapatric subspecies within the yellow mongoose, *C. penicillata*, (1) *C. p. bradfieldi*, (2) *C. p. coombsii*, (3) *C. p. penicillata* and (4) *C. p. natalensis*. Adapted from Taylor (1990).

which occurs over the largest part of South Africa to the exclusion of Kwazulu/Natal, the latter region being limited to *C. p. natalensis*; *C. p. bradfieldi* which is found in Namibia and *C. p. coombsii* which is distributed throughout Botswana and most of the north western reaches of the Northern Transvaal. In this investigation, Taylor (1990) adopted the subspecies definition proposed by Lidicker (1962): "A subspecies is a relatively homogenous and genetically distinct portion of a species which represents a separately evolving, or recently evolved, lineage with its own evolutionary tendencies, inhabits a definite geographical area, is usually at least partially isolated, and may intergrade gradually, although over a fairly narrow zone, with adjacent subspecies". Taylor's (1990) description of the subspecies was based on cranial measurements taken from 734 specimens from 272 localities with the four subspecies being distinguished primarily on the basis of skull size. In a subsequent report Taylor and Meester (1993) were equivocal on the status of *C. p. natalensis*, a decision prompted by the small sample size available from this region. Widespread intergradation occurs between *C. p. bradfieldi* and *C. p. coombsii* while overlap between these two subspecies and *C. p. penicillata* is more restricted (see Fig.1).

The morphometric transition zones between the subspecies tend to coincide with biogeographical and ecological features such as the lower Orange River, the Soutpansberg Mountains and the limits of the Kalahari sands. A hypothesis advanced by Taylor (1990) was that some measure of genetic divergence underlined these morphometrically defined subspecies. To test this, 28 allelic systems were analyzed by starch gel electrophoresis in eight yellow mongoose populations representing two subspecies, *C. p. penicillata* and *C. p. bradfieldi* (Taylor, Campbell, Van Dyk, Watson, Pallett & Erasmus 1990). Thirteen loci were polymorphic with average heterozygosity (H) calculated at 3.4%. Different populations were found to be closely related and showed no correlation with subspecies designations, thus failing to support the morphometric delimitation. However, these authors were of the opinion that the inconsistencies between the morphometric and allozyme data were probably due to the latter's inability

to provide adequate resolution, and that genetic differences nonetheless exist between taxa.

GENERAL BIOLOGY

While a comprehensive survey of the species' biology is clearly peripheral to the main thrust of this investigation, there are certain life history characteristics which are directly applicable to an assessment of genetic population structure. These include habitat fragmentation, reproductive turnover and dispersal patterns.

Habitat discontinuity

The area occupied by any species is the result of a balance between the tendency to broaden its area of distribution and constraining extrinsic factors (Lynch 1980). The yellow mongoose does not occur in deserts, forests and areas of thick bush (Smithers 1983). Excluding these areas, however, it ranges over most of southern Africa (Fig.2), except the northern part of the Eastern Cape, southern and northern Kwazulu/Natal, most easterly reaches of the Eastern and Northern Transvaal, Mozambique as well as the largest part of Zimbabwe (Lynch 1975; Pringle 1977; Smithers and Wilson 1979; Skead 1980; Rowe-Rowe 1992).

Reproduction

Female reproductive patterns in the yellow mongoose have been the subject of several investigations which yielded conflicting results. Snyman (1940) reported that females could produce two litters per year. Both Zumpt (1976) and Lynch (1980) dispute this, finding evidence for only a single litter per breeding season which, in turn, has been questioned by Wenhold (1990) and Rasa, Wenhold, Howard, Marais and Pallett (1992) who support Snyman's (1940) initial observation of two litters per year. Rasa and co-workers (1992) also show that the previous year's young are not forced to disperse with the birth of the new litter, but remain with the group, acting as helpers thus ostensibly promoting breeding site philopatry.

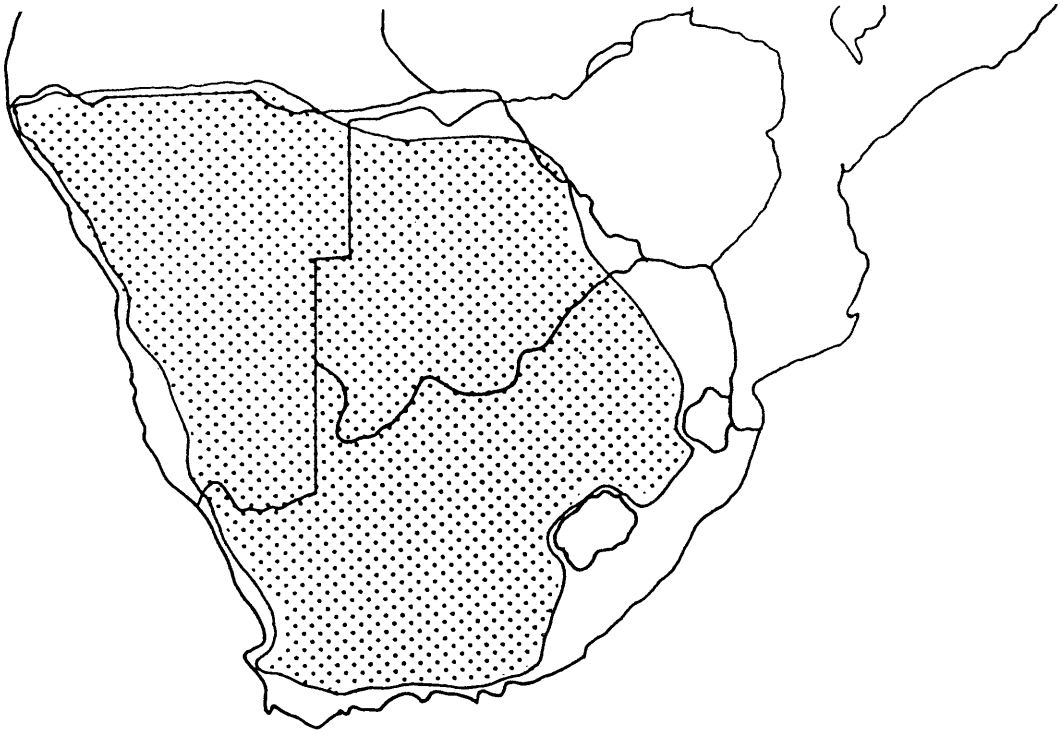

Figure 2: Geographic distribution of the yellow mongoose, *C. penicillata*, redrawn from Skinner and Smithers (1990).

THE YELLOW MONGOOSE AS A RABIES VECTOR

Rabies is a disease that causes high mortality in all warm-blooded vertebrates, where the symptoms include irrational, excitable and furious behaviour, the inability to swallow, salivation, convulsions, and in humans hydrophobia (Swanepoel, Barnard, Meredith, Bishop, Brückner, Foggin & Hübschle 1993). The viral infection is caused by a member of the family Rhabdoviridae (Howell 1982). The saliva of infected animals has been shown to have high virus titres promoting transmission through the bite of a diseased animal.

Cumming (1982) reported that natural rabies cycles are characterized by accommodation between host and virus in such a manner that mortality of the host is sufficiently low to ensure persistence of the virus. In these natural cycles, it is possible for the virus to be present in a population without it being particularly overt since mortality due to rabies is often classified as a natural death. The probability cannot be excluded, however, that pathenogenic viruses, such as the rabies virus, play a role equal to that of predators in determining the success of natural populations: over a long time it can shape the demographic profile of a species (O'Brien & Evermann 1988). There are several potential consequences that are of evolutionary significance when a population/species' numbers are reduced by an epidemic. First, selection will take place for individuals that are genetically more resistant to the virus than their ancestors. Secondly, intense selective pressure will alter the allele frequencies of other loci that are genetically linked to loci affecting resistance. Thirdly, if population's numbers decrease sufficiently, it will cause the population to experience a bottleneck which, in turn, can lead to extinction and subsequent recolonization. Alternatively, inbreeding could result, should some members of the population survive infection (O'Brien & Evermann 1988).

The existence of two main rabies-type areas in southern Africa is acknowledged (Fig.3; Meredith 1982). In the first, the herpestids are the main wildlife reservoirs, with the felids and mustelids as insignificant contributors. The second has the jackal and/or semi-feral

Figure 3: Geographic partitioning, by principle vector, of rabies in southern Africa. Redrawn from King, Meredith and Thomson (1995).

dog as the principal disseminators of the disease. Importantly within the context of the present investigation, *C. penicillata* is regarded as being the main rabies vector within South Africa (Snyman 1940; Meredith 1982).

Rabies outbreaks pose serious problems to health care in modern society and indications are that with increasing poverty and population growth, the situation is likely to worsen. Since the first report of the disease, attempts have been made to eradicate rabies and, by implication, the disease vectors. Researchers agree, however, that the total elimination of the host species will not be feasible, especially in the case of a small carnivore such as the yellow mongoose (Zumpt 1976), and it is therefore essential to explore other options for the containment of the disease. Possible correlations between genetically detected clusters of the yellow mongoose and geographic distributions of the viverrid-type rabies might indicate local susceptibility of the host species to the virus which is perhaps underpinned by some genetic factor(s). In assessing the degree of correspondence between the yellow mongoose's geographic distribution and the area in which the viverrid-type rabies predominates, it is evident that *C. penicillata* acts as a rabies reservoir only within a distinct part of its distributional range. Intriguingly, this corresponds loosely with the borders of *C. p. penicillata* as recognized by Taylor and Meester (1993; see Figs.1 and 3).

MITOCHONDRIAL DNA

Molecular Characteristics and Mode of inheritance:

The mitochondrial DNA molecule is a circular gene system that replicates and transcribes within the organelle itself (Avise & Lansman 1983). Traditionally, gene order within the molecule was thought to be highly conserved and the order has remained unchanged for 350 million years (Barton & Jones 1983). This has, however, recently been questioned by Moritz, Dowling and Brown (1987) and Desjardins and Morais (1990). Apart from the 37 genes coded for by the mitochondrial DNA molecule, there are also two separate non-

coding regions which contain the light and heavy strand promoters as well as the origin of replication for both these strands.

Mitochondrial DNA has been described as the female analogue of male surname transmission (Avise 1991), with very little or no detectable paternal contribution. Unless the paternal contribution of mitochondria to the offspring is at least 1-5% of the total DNA, it often goes unnoticed (Avise & Lansman 1983). Lansman, Avise and Huettel (1983) determined that the upper limit of paternal leakage in *Heliothus* budworms is 1 molecule per 25 000 per generation. Recently, a paternal contribution of 10^{-4} was found in laboratory strains of mice (Gyllensten, Wharton, Josefsson & Wilson 1991), but since this study made use of extensive backcrosses, it might not necessarily reflect a universal phenomenon.

Evolution of mitochondrial DNA:

The discovery of an elevated nucleotide substitution rate for mitochondrial DNA in primates (Brown, George & Wilson 1979; Brown, Prager, Wang & Wilson 1982) was unexpected since it was thought that functional constraints would not permit viable changes to genes (Avise 1991). The elevated mutation rate has enhanced the use of mitochondrial DNA in evolutionary studies. Assuming a constant rate of sequence turnover, estimates of existing amounts of divergence can be used to calculate the time since two individuals\lineages last shared a common female ancestor (Harrison 1989). This assumptions should, however, be viewed with caution. Not only do different parts within the molecule diverge at different rates (Nei 1987; Harrison 1989), but there is also increasing evidence for variation in rates of nucleotide substitution between divergent taxonomic groups (Avise, Bowen, Lamb, Meylan & Bermingham 1992; Martin, Naylor & Palumbi 1992). Comparisons of mitochondrial and nuclear DNA divergence among echinoid and vertebrate taxa of similar ages indicate that the rapid rate of vertebrate mitochondrial DNA evolution is, in part, an artefact of a widely divergent rate of nuclear DNA evolution (Vawter & Brown 1986).

Phylogeography:

The majority of species are composed of geographic populations whose members occupy different branches on an intraspecific phylogenetic tree (Avise, Arnold, Ball, Bermingham, Lamb, Neigel, Reeb & Saunders 1987). Since data from mitochondrial DNA only reflect the matriarchal phylogeny (Avise *et al.* 1987), the historical picture recorded in this molecule is not a complete characterization of the intraspecific phylogeny of a species. Clearly, relationships may be disturbed if males and females differ in phylogeographically relevant characteristics, such as variances in progeny numbers and levels of dispersal (Takahata & Palumbi 1985).

Avise and co-workers (1987) have identified a number of possible outcomes to the study of phylogeographic variation within taxa, ranging from discontinuous to continuous. A mitochondrial DNA phylogeny could show discontinuities or genetic breaks in which arrays of related genotypes differ by a number of mutational steps. Such genetically distinct mitochondrial DNA assemblages might occupy separate geographic regions within the range of a species, or they could co-occur geographically. Alternatively, mitochondrial DNA phylogenies themselves might be genetically continuous, and spatially either disjunct, totally overlapping, or nested.

Equally important, the long-term persistence of only one maternal lineage in a population can be explained by random lineage extinction where the level of diversity of mitochondrial DNA will decrease over time in finite populations (Avise, Neigel & Arnold 1984). A classical example of this would be females that produce only male offspring and this could also be evoked to explain the low levels of variation found in some populations.

In addition to the widespread use of mitochondrial DNA restriction site/fragment data for assessing phylogeographic relationships (O'Brien, Roelke, Yuhki, Richards, Johnson, Franklin, Anderson, Bass, Belden & Martenson 1990; Wayne, Meyer, Lehman, Van Valkenburgh, Kat, Fuller, Girman & O'Brien 1990; Girman, Kat, Mills, Ginsberg, Borner, Wilson, Fanshawe, Fitzgibbon, Lau & Wayne 1993), sequences obtained from the major

non-coding region have also successfully been used to determine population structure within species (Hoelzel & Dover 1991; Wilkinson & Chapman 1991; Graven, Passarino, Semino, Boursot, Santachiara-Benerecetti, Langaney & Excoffier 1995). Given the close association between the morphometrically defined subspecies (Taylor 1990; Taylor & Meester 1993), it was anticipated that mitochondrial DNA clusters would also be separated by low sequence divergences. Consequently, as an adjunct to the mitochondrial DNA RFLP study, analysis of the major non-coding region was also included in the present investigation. While this approach has proved useful in other studies (Hoelzel & Dover 1991; Wilkinson & Chapman 1991; Graven *et al.* 1995), an array of repeats contained within the major non-coding region could, potentially, confound phylogenetic reconstruction. In instances where length variation is known to accompany differences detected with restriction enzymes, more often than not the length differences show little geographic or phylogenetic concordance with cleavage site data (Cann & Wilson 1983; Harrison, Rand & Wheeler 1985).

Length polymorphisms

Length differences in mitochondrial DNA occur frequently among species, representatives of the same species, and even within specimens - a phenomenon called heteroplasmy (Hayasaka, Ishida & Horai 1991; Monforte, Barrio & Latorre 1993; Stewart & Baker 1994). Most length variation is specific to the control region of the mitochondrial DNA (Anderson, Bankier, Barrell, De Bruijn, Coulson, Drouin, Eperon, Nierlich, Roe, Sanger, Schreier, Smith, Staden & Young 1981; Bibb, Van Etten, Wright, Walberg & Clayton 1981). Length differences are usually ascribed to variation in the number of nucleotides in homopolymer tracts, to variation in the copy number of short tandem repeats, and to the duplication or deletion of unique sequences (Monnerot, Mounolou & Solignac 1984; Solignac, Monnerot & Mounolou 1986; Mignotte, Gueride, Champagne & Mounolou 1990).

A number of different mechanisms have been proposed to account for observed length differences, for example the competitive displacement model (Buroker, Brown, Gilbert, O'Hara, Beckenbach, Thomas & Smith 1990), slip-mispairing events in tandem arrays

(Efstratiadis, Posakony, Maniatis, Lawn, O'Connell, Spritz, Deriel, Forget, Weissman, Slightom, Blechi, Smithers, Baralle, Shoulders & Proudfoot 1980; Levinson & Gutman 1987), and unequal crossing over (Smith 1976 in Hoelzel 1993). There are three types of length variation: first, insertions or deletions of only a few nucleotides (Monnerot *et al.* 1984), second, variation in the copy number of tandem repeats (Mignotte *et al.* 1990) and lastly, duplication or deletion events involving large regions of the genome (Moritz & Brown 1987).

In examining different length polymorphisms, it becomes clear that it is often caused by tandem arrays which are repeated a variable number of times (VNTR). It is not always clear, however, whether smaller or larger fragment sizes are favoured, although smaller mitochondria have an advantage when it comes to inheritance from mother to offspring (Rand & Harrison 1986). This phenomenon is explained by the so-called "race for replication" where shorter mitochondrial DNA molecules will complete replication faster than longer ones (Hale & Singh 1991). Rand (1993) showed that homeotherms tend to have smaller mitochondrial genomes than poikilotherms and ascribe this to the higher metabolic demands associated with endothermy which, in turn, impose a stronger purifying selection in the cytoplasm of endotherms. However, the tendency for shorter mitochondrial genomes may be questioned by the existence of high numbers of copies of a direct repeat, for example up to eight repeat sequences, in the evening bat (*Nycticeius humeralis*; Wilkinson & Chapman 1991).

AIMS

Traditionally, studies dealing with the taxonomic status of subspecies relied on phenotypic traits such as coloration and size. These are, however, strongly influenced by environmental factors which were often reflected in the taxonomic placement of groups. The use of molecular markers, such as the mitochondrial DNA molecule which is free of environmental influences, can often provide a robust means of assessing phylogeographic structure and detecting possible genetic provinces within species.

The main objectives were:

1. To undertake a characterization of mitochondrial DNA variability and differentiation among geographic samples of the yellow mongoose throughout its distributional range.

2. To determine whether unique foci of endemic rabies outbreaks correlate with discrete geographic subdivisions of the *C. penicillata* mitochondrial DNA gene tree.

CHAPTER 2
MATERIALS AND METHODS

MITOCHONDRIAL DNA RFLP STUDY

Sample collection and storage

The mitochondrial DNA RFLP analysis reported herein encompassed 50 specimens drawn from 21 populations, representing all the morphometric subspecies delineated by Taylor (1990) and Taylor and Meester (1993). The map positions of the collection localities are shown in Figure 4. Map co-ordinates for all collection sites are listed in Appendix 1.

Specimens were either shot, or live trapped, and subsequently sacrificed with Euthanaze solution (Centaur labs; 1 ml/1 kg body mass). Body measurements were taken if possible, and when available, skulls and skins were placed in the mammal collections of the Transvaal Museum (Pretoria), National Museum (Bloemfontein), Durban Natural Science Museum (Durban) and the McGreggor Museum (Kimberley). Museum accession numbers are presented in Appendix 2.

Ultrafreezing is a reliable method for the long term preservation of soft tissue. The heart, kidneys and liver of specimens were removed and immediately frozen in liquid nitrogen. Since freezing facilities were not always available, as an alternative, the tissue was cut into thin slices and placed into a saturated salt:20% dimethylsulfoxide (DMSO) solution (Amos & Hoelzel 1991); the salt has preserving properties (denatures proteins) while DMSO enhances cell membrane permeability.

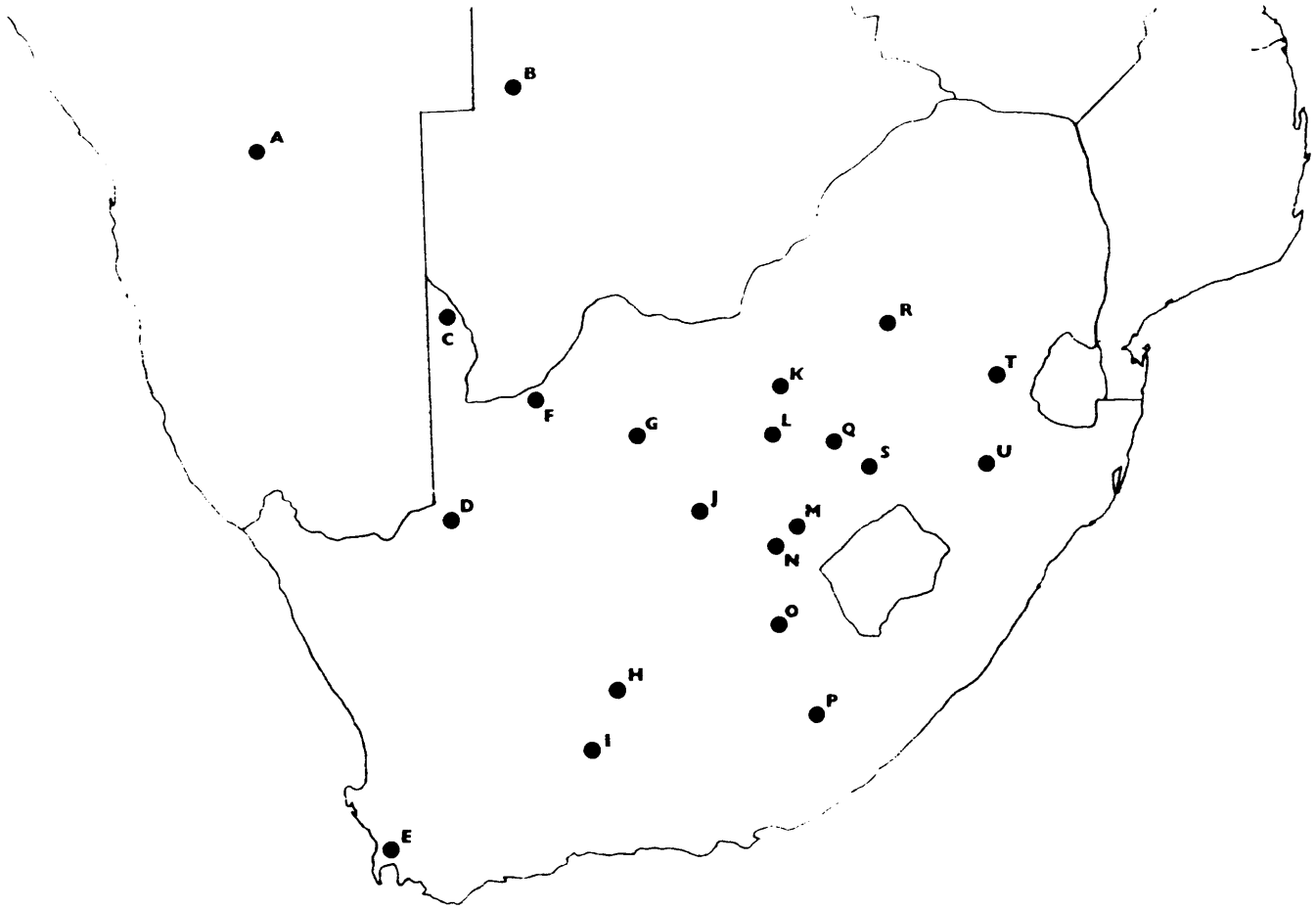

Figure 4: Twenty one collection localities selected to cover the geographic distribution of the yellow mongoose, *C. penicillata* in southern Africa. Namibia: Windhoek (A). Botswana: Ghanzi (B). South Africa: Kalahari Gemsbok National Park (C), Riemvasmaak (D), Stellenbosch (E), Van Zylsrus (F), Kuruman (G), Victoria West (H), Karoo National Park (I), Kimberley (J), Baberspan Nature Reserve (K), Sandveld Nature Reserve (L), Bloemfontein Glen (M), Bloemfontein (N), Tussen-die-Rivieren Game Farm (O), Queenstown (P), Kroonstad (Q), Pretoria (R), Willem Pretorius Nature Reserve (S), Ermelo (T), Chelmsford Nature Reserve (U). Corresponding map co-ordinates are presented in Appendix 1.

Mitochondrial DNA extraction and purification

Mitochondrial DNA was extracted from soft tissue using a standard protocol (Lansman, Shade, Shapira & Avise 1981). This extraction procedure requires fairly large amounts of soft tissue (2-10 g). The heart of the yellow mongoose often weighed less than 2 g and in these instances the source material was supplemented with kidney tissue.

In brief, the extraction procedure involved the removal of cell debris and proteins from the sample, followed by the establishment of the density gradient by the addition of cesium chloride to a final concentration of 1.56-1.60 g/ml. To visualize the mitochondrial DNA band under a UV light, 600 μl (200 μl/3 ml suspension) ethidium bromide (10 mg/ml) was added to each tube. Tubes were capped and the gradient overlaid with liquid paraffin to prevent tube collapse. Following 36-48 h of ultracentrifugation (Beckman L7-55 Preparative Ultra-centrifuge), the fractionized samples were visualized under UV-light and the mitochondrial DNA band removed using a sterile 18 G needle and syringe. In instances where a mitochondrial band was not visible, the gradient approximately 1 cm below the nuclear band was taken. Ethidium bromide was removed from the sample by the addition of an equal volume of iso-amylalcohol, and the cesium chloride dialysed against an excess $T_{10}E_{0.5}$ (0.01 M Tris-base, 0.5 mM EDTA; pH8.0).

Total genomic extractions

The combined amount of soft tissue available for some of the yellow mongoose specimens did not allow the extraction of good quality mitochondrial DNA, and in these instances total genomic chromosomal DNA was extracted following Amos and Hoelzel (1991). The same procedure was used for the extraction of total genomic DNA from soft tissue preserved in saturated salt:20% DMSO solution. This requires between 0.1 and 0.3 g of starting tissue, and from this yields of approximately 3 μg/μl were obtained.

Tissue was quick-frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. The powder was suspended in a digestion solution (50 mM Tris, 1% SDS, 10 mM EDTA, 100 mM NaCl) and incubated at 55°C for 4-6 h. RNase was added to a final

concentration of 20 μg/ml. The DNA was purified by phenol extractions and the phenol subsequently removed by an equal volume of chloroform:iso-amylalcohol. Proteins were removed by centrifugation and the DNA ethanol precipitated. Depending on the size of the dried mitochondrial DNA pellets, they were suspended in 80-100 μl $T_{10}E_{0.5}$.

Restriction endonuclease digestion of DNA

Eighteen type II restriction endonucleases were employed in the mitochondrial DNA RFLP study (Table 1). This type of restriction endonuclease cuts DNA within the specific recognition sequence with a twofold axis of symmetry (Maniatis, Fritsch & Sambrook 1982). The 18 six-base recognition restriction endonucleases were selected on their ability to cleave the yellow mongoose mitochondrial genome, regardless of the number of recognition sites and hence fragments produced.

Mitochondrial and total genomic DNA was digested with each of the 18 endonucleases following conditions recommended by the manufacturers (Amersham; Boehringer Mannheim; Promega). Digestions were carried out in 20 μl reaction volumes containing 12-25 units of enzyme, 1 x buffer and 2 mM spermidine per reaction volume; these were incubated overnight at 37°C.

Endlabeling of mitochondrial DNA

Mitochondrial DNA restriction fragments were endlabeled to high specificity using 0.25 μCi of either [α-^{32}P] dATP or -dCTP (Amersham International plc, Amersham UK; ICN). The large Klenow fragment of *Escherichia coli* was used for incorporating the labeled and unlabeled (cold) nucleotides (Brown 1980). After terminal bases of the template were removed and subsequently filled in with either radio-active isotope, or one of the other cold nucleotides, the reaction was stopped by heating to 65°C.This was followed by the addition of 3 μl of tracking dye. (50% glycerol, 0.1% bromophenol blue, 0.1% EDTA).

Table 1: Restriction endonucleases employed in the present study with their respective recognition sequences. The r-values are from Nei (1987).

Restriction endonuclease	Recognition sequence	r-value
Apa I	GGGCC\C	6
Asp 718	G\GTACC	6
Ava I	C\(T/C)CG(A/G)G	5.3
Bam HI	G\GATCC	6
Bcl I	T\GATCA	6
Cla I	AT\CGAT	6
Dra I	TTT\AAA	6
Eco RI	G\AATTC	6
Eco RV	GAT\ATC	6
Hind III	A\AGCTT	6
Kpn I	GGTAC\C	6
Pvu II	CAG\CTG	6
Sac I	GAGCT\C	6
Sca I	AGT\ACT	6
Sfu I	TT\CGAA	6
Stu I	AGG\CCT	6
Xba I	T\CTAGA	6
Xho I	C\TCGAG	6
Alu I	AG\CT	4
Hae III	GG\CC	4
Hinf I	G\ANTC	4
Hpa II	C\CGG	4
Rsa I	GT\AC	4
Taq I	T\CGA	4
Tru 91	T\TAA	4

Gel electrophoresis of mitochondrial and total genomic DNA

Separation of endlabeled mitochondrial DNA fragments was done using horizontal 1% TBE buffered agarose gels (0.08 M Tris base, 0.089 M Boric acid, 0.002 M EDTA; pH8.0) at 1.5-2.0 V\cm. Lambda phage digested with Hind III (Promega marker II) was used as a molecular weight standard. To resolve some of the smaller fragments, samples were electrophoresed on a 2% agarose gel. The agarose gels were vacuum dried onto 3 MM Whatman chromatography paper using a slab gel dryer (Hoefer Scientific Instruments). During this process, the temperature was kept constant at 54°C for the first hour to facilitate the melting of the agarose.

Digested total genomic DNA fragments were electrophoresed on 0.8-1.0% agarose gels containing ethidium bromide, and transferred onto Hybond-N nylon membranes (Amersham; Southern 1975). In short, digested fragments were denatured (1.5 M NaCl, 0.5 M NaOH) to ensure the transfer of single stranded DNA, and neutralized (1.5 M NaCl, 0.001 M EDTA, 0.5 M Tris base; pH7.2). The membranes were rinsed in 10 x SSC (0.3 M NaCl, 0.03 M tri-sodium citrate; pH7.0) after which capillary blots were prepared using standard procedures (Maniatis *et al.* 1982) with 10 x SSC as the transfer buffer. Membranes were baked at 80°C for 2-3 h to bind fragments to them.

Oligolabeling of the mitochondrial DNA probe

Purified mitochondrial DNA (100 ng) was oligolabeled to a high specific radioactivity (10^7-10^8 cpm) with [α-^{32}P] dATP using the Klenow fragment (Feinberg & Vogelstein 1983). The labeled DNA was subsequently spermine precipitated to separate it from unincorporated nucleotides. Salmon sperm DNA (8 µl of a 10 mg/ml stock) acted as carrier DNA to prevent loss of the labeled probe. The DNA was pelleted, and 2 µl of the supernatant retained to determine the percentage incorporation. The DNA pellet was incubated for 30 min in the presence of 0.5 M NaCl in $T_{10}E_{0.1}$ and 4 N NaOH to chemically denature the DNA. Probe DNA was subsequently neutralized with 2 M Tris and 2 µl of the precipitated probe retained for determining the specific radioactivity using a Packard 1500 tri-carb Liquid Scintillation Counter.

Blot hybridization

Hybridization of labeled mitochondrial DNA to the membrane bound fragments is dependent on both of the DNA species (probe DNA and membrane-bound fragments) being single stranded as well as the degree of homology between them. Before the addition of the probe DNA, membranes were pre-hybridized (100 mM Denhardts, 10 x SSPE, 10% SDS) to saturate the binding sites on the membrane in an attempt to reduce non-specific background (Mason & Williams 1985). Hybridization was carried out overnight at 65°C. Both pre- and post -hybridization steps were performed in sealed plastic bags.

Unincorporated nucleotides and mismatched hybrids were removed by a single low stringency wash (2 x SSPE, 0.1% SDS). In the case of the yellow mongoose, this 15 min wash was sufficient to remove excess nucleotides. SDS was removed by rinsing in 0.1 x SSPE after which the membranes were resealed in plastic bags.

Fragment detection and developing of autorads

Fragments were detected using Okomoto X-ray cassettes and Fugi RX medical X-ray film. The cassettes were kept in an ultracold freezer until photographic development with exposure time dependant on the radio activity of the blots (2-36 h). Autoradiographs were developed (Ilford Phenisol) and fixed (Ilford Hypam) using standard procedures.

MITOCHONDRIAL DNA RFLP DATA INTERPRETATION AND ANALYSIS

Restriction endonucleases detect differences in base composition between specimens which, in turn, generate differences in restriction profiles. Unique fragment patterns produced by the different restriction endonucleases were assigned lower case alphabetical letters resulting in each specimen being characterized by an 18 character profile. Each unique mitochondrial DNA profile (composite haplotype) represents a distinct maternal lineage which, in turn, was designated by an upper case alphabetical letter.

Quantitative analysis

Estimation of the number of nucleotide substitutions in the yellow mongoose was done using the Restsite computer package (v1.1) of Nei and Miller (1990). The estimates of sequence divergence are based on the formulas of Nei and Li (1979). The proportion of shared fragments between lineages (F) can be calculated by

$$2n_{xy}/(n_x+n_y)$$

where n_x and n_y are the number of fragments in population X and Y respectively, and n_{xy} is the number of shared fragments between the two populations. The number of nucleotide substitutions per site can be determined using

$$-(2/r)\log_e G.$$

G is determined by

$$[F(3-2G_1)]^{1/4}$$

where G_1 is a trial value for G. This iterative computation is done until $G=G_1$ is obtained.

To correct for multiple hits at the same site the Jukes and Cantor (1969) correction factor was applied. This assumes that nucleotide substitutions occur at any site with equal probability, and that at any given site a nucleotide can change to one of the three remaining sites with a rate of λ per year. The Jukes and Cantor (1969) correction is also most accurate for small values of d (Nei 1987). The correction is applied using

$$d=-\tfrac{3}{4}\log_e(1-4/3p)$$

where p=1-q is the proportion of different nucleotides between population X and Y.

The nucleon diversity (Nei & Tajima 1981), or genotypic diversity (Avise, Bowen & Lamb 1989), indicates the frequency of occurrence of the different maternal lineages in a population. Genotypic diversity can be estimated by

$$n(1-\sum f_i^2)/n-1$$

where f_i is the frequency of the i^{th} mitochondrial DNA genotype in a sample of size n. The genotypic diversity value varies between 0 (when all the individuals exhibit the same genotype) and 1 (when each individual displays a unique composite haplotype).

Qualitative analysis

The genetic diversity present within the yellow mongoose was graphically displayed using a parsimony generated mitochondrial DNA phylogeographic network (Avise, Lansman & Shade 1979). The number of base substitutions, as detected by the suite of restriction endonucleases, was calculated between composite matrilines and the resulting data overlaid onto a map of southern Africa. This approach is based on the assumption that complex digestion pattern phenotypes cannot be the result of convergent evolution from unrelated phenotypes; thus specimens sharing multiband patterns after digestion of mitochondrial DNA can be unambiguously assigned to a common evolutionary origin.

Phenogram construction

The construction of trees based on the yellow mongoose data set were done using two methods. The first is the cross-averaging UPGMA (unweighted pair-group method using arithmetic averages; Sneath & Sokal 1973). This method constructs a tree based on the analysis of the similarity matrix. The two taxa with the lowest similarity coefficient in a distance matrix are grouped (or alternatively the highest sequence divergence in a correlation matrix), the average coefficient for the two clustered taxa determined, and the cluster thus formed is then treated as a single unit in a new matrix (Swofford & Olsen 1990, Mayr & Ashlock 1991). This method assumes that the data are both additive and ultrametric.

The Neighbour-Joining (NJ) method was also employed (Saitou & Nei 1987); the NJ algorithm builds the phylogeny according to a rigid stepwise procedure and subsequently minimizes the total length of the tree at each step (DeBry 1992). This method operates under the assumption that the lengths of the branches lying on the path between any pair of terminal taxa can be summed to yield a meaningful quantity. It does, however, not assume that the data are ultrametric. Jin and Nei (1991) showed that for low sequence divergences, the NJ method produced the correct phylogeny with a higher probability than the UPGMA method. This was ascribed to the fact that the UPGMA tree's accuracy relies on the assumption of an equal rate of nucleotide substitution, a criterion which is not always met.

MITOCHONDRIAL DNA CONTROL REGION ANALYSES

Polymerase chain reaction (PCR)

Approximately 600 bp on the 5' side of the control region was amplified by the Polymerase Chain Reaction (PCR; Mullis & Faloona 1987) using primers specifically designed for carnivores (Dr. R.K.Wayne, pers.comm.). The L primer (THRL1; 5' GAATTCCCCGGTCTTGTAAAC-C 3') is situated in the $tRNA^{Thr}$ and corresponds to position 16245 of the harbor seal mitochondrial control region, while the H primer (DLH1; 5' CTTGAAGTAGGAACCAGAT-G 3') is situated in the relatively conserved central block (Fig.5a; See text below for discussion of PCR primer DLL1) and corresponds to the complimentary sequence of the L strand position 16750 of the same species (Árnason & Johnsson 1992).

PCR reactions were set up in a laminar flow hood to minimize the risk of contamination and a negative control, comprising all components except DNA, was invariably included to test for cross-contamination. Amplification was performed in 50 μl reaction volumes

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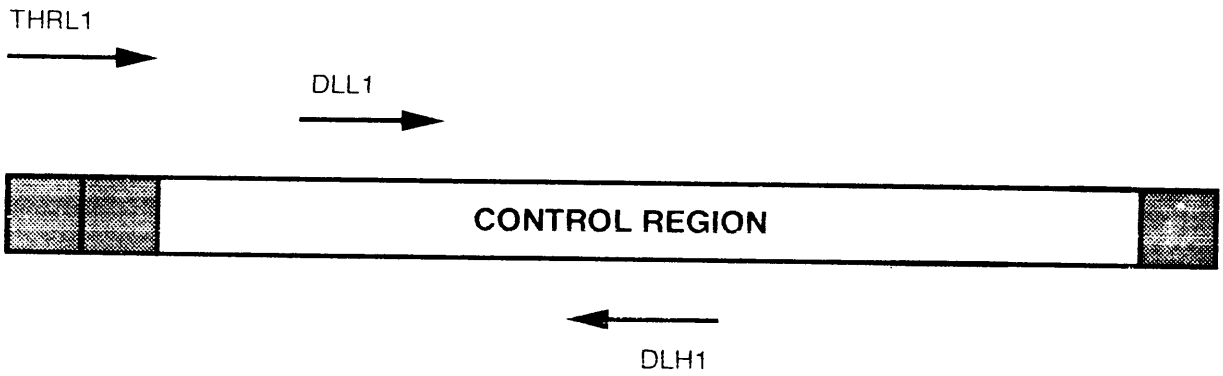
(a)



(b)

```
B4          TACTGTGCTTGCCCAGTA
B13         ******************
B24         ******************
B25         ******************
B37         **********T*******
DL-CLU24    *********ATGT*****
DL-CAU      ****A****ATGT*****
DL-PVI      *********AT*A*****
```

Figure 5: A schematic representation (a) of the major non-coding region and flanking regions of the mitochondrial DNA genome. The relative positions of the primers (THRL1, DLL1 and DLH1) which were used for the amplification and sequencing of this region are also shown. Figure 5 (b) shows the nucleotide sequence alignment for all species used in designing the primer (DLL1). B4-B37 = *Cynictis penicillata*, DL-CLU24 = *Canis lupus*, DL-CAU = *Canis aureus* and DL-PVI = *Phoca vitulina*.

in the presence of 2 units of Dynazyme polymerase, 2 mM dNTP, 50 pmol of each primer and 1 x Dynazyme polymerase buffer (20 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 0.1% Triton X-100, 160 μg/ml BSA and 50% glycerol) in a Hybaid thermal reactor (Hybaid). A 35 cycle program was employed to generate double stranded product of the target species. The cycling parameters were: denaturation for 45 sec at 94°C, annealing for 45 sec at 50°C, and extension for 1 min at 72°C. A final extension cycle of 5 min at 72°C completed the reaction.

Restriction fragment digestion of PCR amplified products

To determine the variation in the major non-coding region of the yellow mongoose, seven four-base recognition restriction endonucleases (Table 1) were employed. Ten μl of the PCR amplified DNA was digested with 5 units of enzyme and digested fragments separated using polyacrylamide gel electrophoresis (PAGE). To ensure separation of both small and larger fragments, digestions were electrophoresed on low (6-8%) and high percentage gels (12-20%). A marker of known fragment lengths (Phi174 digested with Hae III, Promega) was run concurrently with digested samples. Fragment sizes were calculated and differences detected between specimens were scored. PCR products were electrophoresed through 2% low melting agarose gels (NuSieve, FMC Bioproducts). The fragment were excised and purified using commercial purification kits (Magic Prep DNA purification system, Promega; Clean mix, Talent).

Sequencing with chain terminating inhibitors

Sequencing followed Sanger, Nicklen and Coulson (1977). Purified double stranded product was sequenced using the Sequenase Kit (United States Biochemical Corporation). The sequencing reaction consisted of two steps: the annealing step which extends the sequencing primer by adding in deoxytriphosphates and a termination step where the high concentration of dideoxy-triphosphates ensures the termination of strand elongation. The labeling agents were either [α-^{32}P] or -[^{35}S] dATP.

To ensure sufficient overlap between the two strands, a customized primer was designed (DLL1). Major non-coding region sequences were aligned (Fig.5b) for all available yellow mongoose specimens, two canid species (*Canis aureus* and *C. lupus*; Dr. R.K. Wayne unpublished data) as well as the harbor seal (*Phoca vitulina*; Árnason & Johnsson 1992). A region that showed a high degree of homology between all aligned species, and where the G+C content approximated 50%, was chosen for the primer sequence. The DLL1 primer (5' TACTGTGCTTGCCCAGTA-T 3') corresponds to position L16421 of the harbor seal mitochondrial DNA (Árnason & Johnsson 1992). The sequences were electrophoretically separated on 6-7% polyacrylamide gels. An adjustable vertical sequencing system (Hybaid) and Consort E734 power supply was used. To ensure that the DNA was single stranded when loaded onto the gels, samples were heated to 80°C before loading; during the run the DNA was kept single stranded by the urea in the gel mixture as well as by the high percentage of formamide in the sample (Maniatis *et al.* 1982). Gels were transferred onto 3 MM Whatman chromatography paper while those labeled with [^{35}S] dATP were fixed (10% methanol, 10% acetic acid) and vacuum dried onto 3 MM Whatman chromatography paper using a slab gel dryer (Hoefer Scientific Instruments). Fragments were detected using Okomoto X-ray cassettes and Fugi RX medical X-ray film. Autoradiographs were developed (Ilford Phenisol) and fixed (Ilford Hypam) using standard procedures.

Both strands of the mitochondrial DNA fragment were sequenced to score sequences close to the sequencing primers and to allow for the unambiguous scoring of bases in the region of overlap. All specimens used for this analysis were amplified and sequenced more than once for accuracy. Yellow mongoose sequences were deposited in GenBank under Accession Numbers U28425 - U28429.

PHYLOGENETIC ANALYSIS

The control region sequences from five yellow mongoose specimens (subset selection based on the PCR amplification results; see below) were aligned to the corresponding sequences for *C. aureus* and *C. lupus* (Dr. R.K. Wayne unpublished data; Appendix 3). Multiple sequence alignments were done with the computer based alignment program DAPSA (Harley 1994) and corrected by hand. Regions where sequences could not be unambiguously aligned were excluded from subsequent analyses (positions 283 to 364; Appendix 3). The two canid species were specified as outgroups so that character states could be polarized and the resultant tree(s) rooted. Outgroup selection for the parsimony analysis was constrained by the availability of carnivore non-coding region sequences.

Phylogenetic analysis using PAUP (Phylogenetic Analysis Using Parsimony, version 3.1.2d5 Swofford 1993) and PHYLIP (Phylogenetic Inference Package, version 3.5c Felsenstein 1993) were employed for the construction of phylogenetic trees. Sites were treated as unordered characters with four possible character states (G, A, T or C).

CHAPTER 3
RESULTS

RFLP ANALYSIS

The mitochondrial DNA genome size of *C.penicillata* was calculated at 17.7 kb ± 108 bp. Since the scoring of large fragments is subject to error, this estimate is based on enzyme patterns that yielded fragments smaller than 9 kb in size. The average number of fragments examined per specimen was 67.04, resulting in a survey of 402.24 bp or 2.3% of the total mitochondrial genome. The size of the yellow mongoose mitochondrial genome lies somewhat above the 16.8 kb reported for other carnivore species (Wayne, Van Valkenburgh, Kat, Fuller, Johnson & O'Brien 1989; O'Brien *et al.* 1990; Wayne *et al.* 1990; Wayne & Jenks 1991; Wayne, Lehman, Allard & Honeycutt 1992; Mercure, Ralls, Koepfli & Wayne 1993) but well within the range observed in mammals (15.7 to 19.5 kb; Brown 1983).

The majority of restriction endonucleases were monomorphic with eight yielding polymorphic fragment patterns (Fig.6). These consisted of either two or three different restriction profiles obtained for the 50 specimens analyzed. Not all the restriction fragment changes were additive, and the number of site changes between profiles sometimes had to be inferred.

The suite of restriction endonucleases employed revealed 13 maternal lineages (Table 2). These composite haplotypes are distinguished from each other by a number of structural nucleotide changes (mutational steps; Table 3) and the relationships between them summarized in a parsimony network (Fig.7) where the F haplotype is shown to form the hub. Single mutational steps separate most of the haplotypes, indicating recent common ancestry.

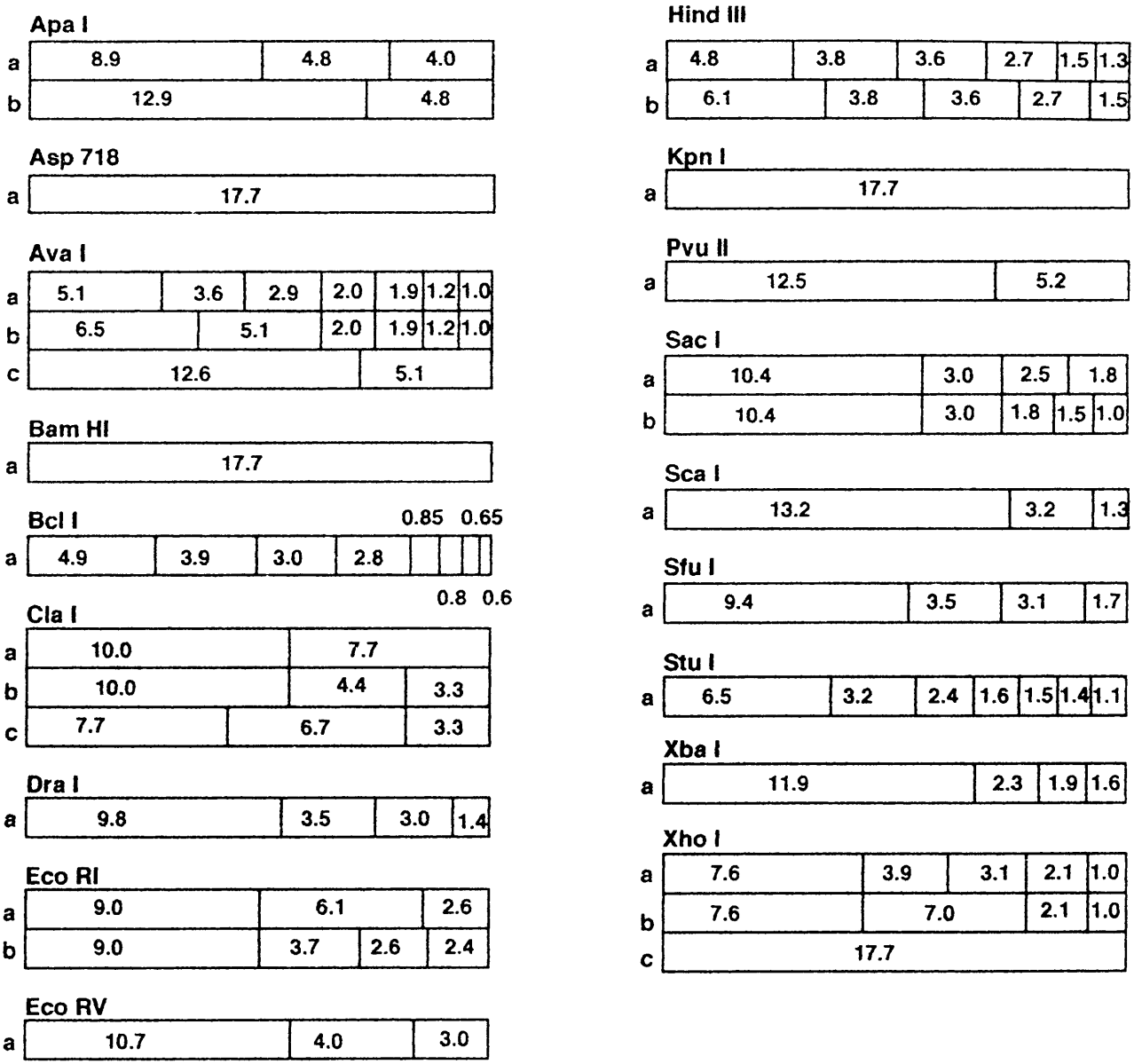


Figure 6: Graphic representation of the fragment composition of restriction profiles identified from yellow mongoose mitochondrial DNA. Fragment sizes are scaled so that their sum equals the overall mean mitochondrial genome size.

Table 2: Thirteen unique maternal lineages (A - M) were identified in the 50 specimens analyzed with 18 restriction endonucleases. Lower case alphabetical letters correspond to the fragments for the different restriction endonucleases presented in Fig.6.

Specimen number	Haplotype	Fragment pattern
B1	A	1b2a3a4a5a6b7a8a9a10a11a12a13a14a15a16a17a18a
B2	B	1a2a3a4a5a6a7a8a9a10a11a12a13b14a15a16a17a18b
B3	C	1b2a3a4a5a6b7a8a9b10a11a12a13a14a15a16a17a18a
B4	D	1a2a3c4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B5	B	1a2a3a4a5a6a7a8a9a10a11a12a13b14a15a16a17a18b
B6	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B7	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B8	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B9	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B10	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B11	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B12	G	1a2a3b4a5a6a7a8a9b10a11a12a13a14a15a16a17a18a
B13	H	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18c
B14	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B15	I	1a2a3b4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B16	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B17	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B18	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B19	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B20	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B21	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B22	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B23	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B24	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B25	J	1b2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B26	J	1b2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a

Table 2 continued

Specimen number	Haplotype	Fragment pattern
B27	J	1b2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B28	J	1b2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B29	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B30	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B31	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B32	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B33	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B34	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B35	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B36	K	1a2a3a4a5a6a7a8b9a10b11a12a13a14a15a16a17a18a
B37	L	1a2a3b4a5a6c7a8a9a10a11a12a13a14a15a16a17a18b
B38	L	1a2a3b4a5a6c7a8a9a10a11a12a13a14a15a16a17a18b
B39	L	1a2a3b4a5a6c7a8a9a10a11a12a13a14a15a16a17a18b
B40	M	1a2a3b4a5a6a7a8a9a10a11a12a13a14a15a16a17a18b
B41	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B42	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B43	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B44	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B45	F	1a2a3a4a5a6a7a8a9a10a11a12a13a14a15a16a17a18a
B46	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B47	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B48	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B49	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a
B50	E	1a2a3a4a5a6a7a8a9a10b11a12a13a14a15a16a17a18a

1:Apa I, 2:Asp 718, 3:Ava I, 4:Bam H1, 5:Bcl I,
6:Cla I, 7:Dra I, 8:Eco RI, 9:Eco RV, 10:Hind III,
11:Kpn I, 12:Pvu II, 13:Sac I, 14:Sca I, 15:Sfu I,
16:Stu I, 17:Xba I, 18:Xho I.

Table 3: Pairwise estimates of the number of mutational steps separating the different composite haplotypes detected in the yellow mongoose.

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	-	4	2	6	3	2	4	6	3	1	4	5	4
B		-	6	6	3	2	4	4	3	3	4	3	2
C			-	8	5	4	6	8	5	3	6	7	6
D				-	5	4	4	8	3	5	6	5	4
E					-	1	1	5	2	2	1	4	3
F						-	2	4	1	1	2	3	2
G							-	6	1	3	2	3	2
H								-	5	5	6	5	4
I									-	2	3	2	1
J										-	3	4	3
K											-	5	4
L												-	1
M													-

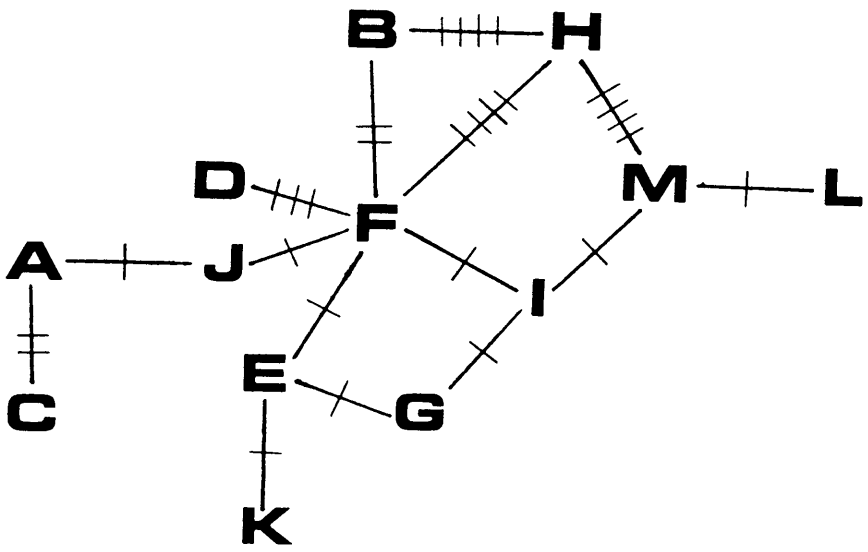


Figure 7: Parsimony network summarizing the relationship between the different composite haplotypes detected in this study. The minimum number of mutational steps separating the haplotypes are indicated as cross-bars on the solid connecting lines and correspond to those in Table 3.

Pairwise estimates of nucleotide sequence divergence among the maternal lineages are presented in Table 4, and the relationships between them summarized in a NJ tree (Fig.8). The topology of the UPGMA tree generated from the same data set was, with minor branch swopping, identical (data not shown; see Jin & Nei 1991 for justification). The C and D haplotypes were the most divergent and are separated by 1.213% (± 0.836%) sequence divergence. The distinctness of these two maternal lineages is reflected in their position on the NJ tree and their respective branch lengths.

Relationships between the different geographic populations are summarized in an UPGMA tree (Fig.9) and the corresponding nucleotide diversity values presented in Table 5. Cluster analysis, based on populations, revealed three major groups even though the percentage divergence between them is low. The geographic affinities of the three assemblages are shown in Figure 10. The Chelmsford Nature Reserve population, the only representative population of *C. p. natalensis*, is the most divergent being separated from the other populations by 0.58% sequence divergence.

Figure 11 shows the geographic overlay of the network reflecting the least number of mutational steps between the different maternal lineages. All the haplotypes, with the exception of E and F, were found to be locality specific. Strikingly, the E haplotype was found to be most frequent (50% of the specimens is characterized by it). The genotypic diversity value of the species was calculated at 0.724. This is relatively low (see Avise *et al.* 1989) and could be attributed to the large number of specimens that display either the E or F haplotype (66%). The genotypic diversity value obtained for *Cynictis* is similar to that obtained for the red-winged blackbird (0.810), a species with prodigious dispersal capabilities (Ball, Freeman, James, Bermingham & Avise 1988). This avian species is characterized by an absence of any real genetic structure.

Table 4: Pairwise estimates of nucleotide sequence divergence (%) between the 13 different yellow mongoose mitochondrial DNA lineages above the diagonal; standard errors derived by bootstrap analysis are presented below the diagonal. The Jukes and Cantor (1969) correction factor was applied to all the values to correct for multiple hits at the same position.

	A	B	C	D	E	F	G	H	I	J	K	L	M
A	-	0.574	0.186	0.987	0.428	0.277	0.589	0.595	0.437	0.137	0.574	0.634	0.427
B	0.286	-	0.799	0.987	0.428	0.277	0.589	0.387	0.437	0.427	0.574	0.437	0.137
C	0.182	0.382	-	1.213	0.644	0.481	0.810	0.830	0.645	0.334	0.799	0.857	0.643
D	0.640	0.655	0.836	-	0.836	0.697	0.720	1.022	0.571	0.846	0.987	0.863	0.846
E	0.229	0.210	0.322	0.575	-	0.138	0.164	0.443	0.302	0.283	0.138	0.594	0.283
F	0.200	0.181	0.320	0.492	0.127	-	0.297	0.287	0.157	0.137	0.277	0.437	0.137
G	0.255	0.208	0.375	0.556	0.141	0.168	-	0.610	0.139	0.444	0.297	0.433	0.444
H	0.342	0.279	0.477	0.654	0.341	0.304	0.346	-	0.452	0.442	0.595	0.553	0.240
I	0.243	0.198	0.306	0.521	0.167	0.140	0.132	0.303	-	0.297	0.437	0.281	0.297
J	0.148	0.240	0.228	0.639	0.189	0.128	0.241	0.341	0.214	-	0.427	0.589	0.282
K	0.258	0.223	0.371	0.666	0.133	0.184	0.191	0.350	0.199	0.231	-	0.737	0.427
L	0.311	0.209	0.399	0.545	0.257	0.245	0.215	0.328	0.219	0.258	0.273	-	0.297
M	0.251	0.147	0.319	0.651	0.186	0.130	0.206	0.225	0.178	0.201	0.208	0.201	-

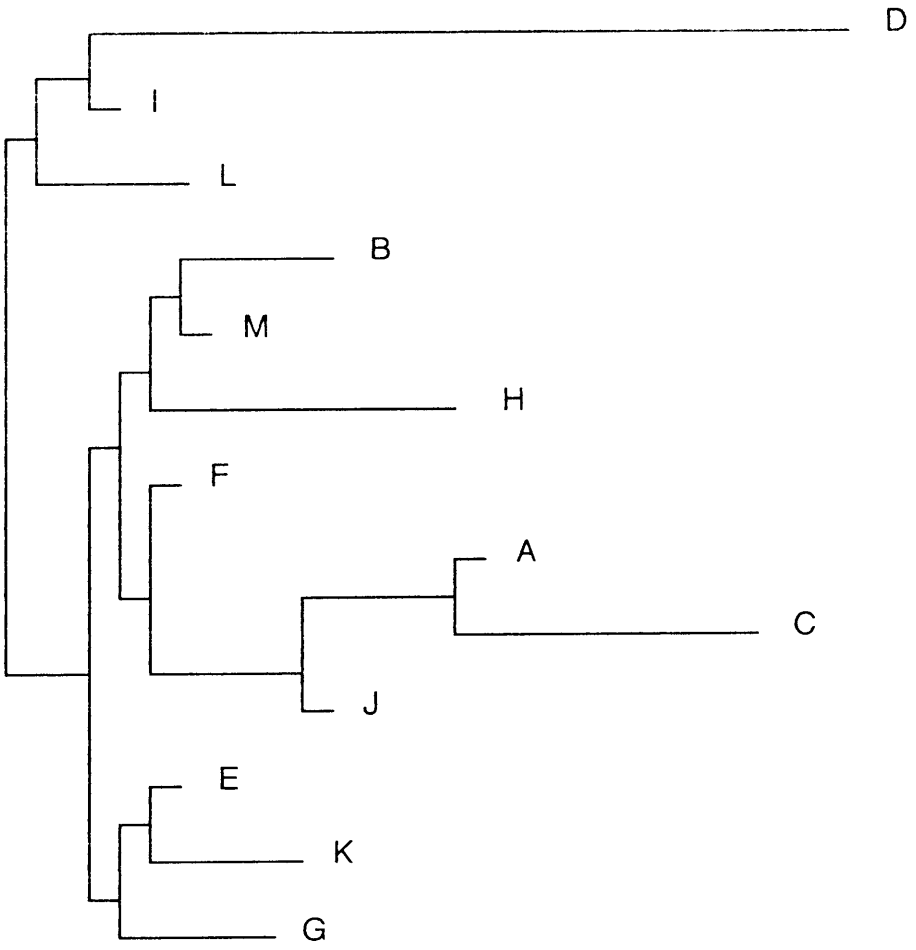


Figure 8: NJ tree summarizing the relationship between the different yellow mongoose maternal haplotypes as detected by RFLP analysis.

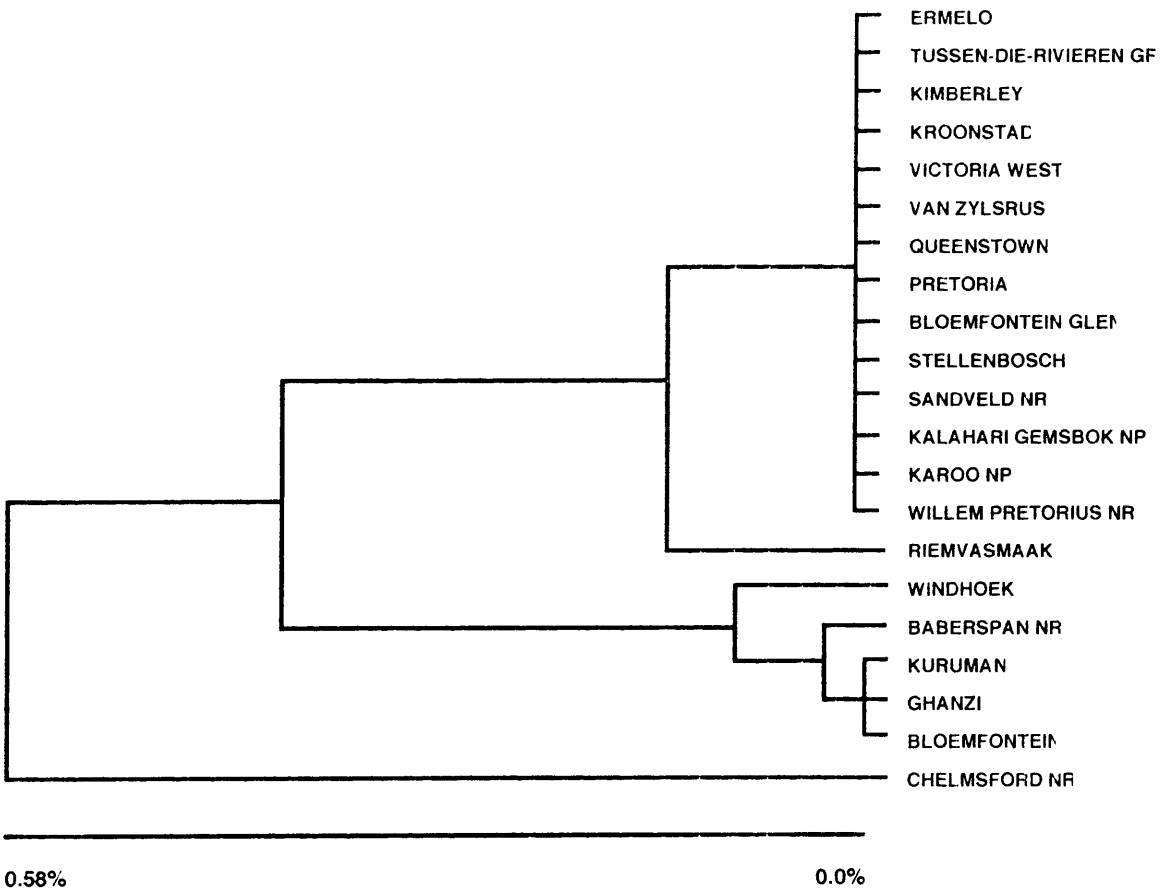


Figure 9: UPGMA tree summarizing the relationship between the 21 yellow mongoose populations sampled in the present study.

Table 5: Nucleotide diversities within the 21 *C. penicillata* populations sampled. A zero value indicates that all specimens are characterized by the same mitochondrial haplotype whilst the absence of a value denotes a single specimen.

Population	Nucleotide diversity	Number of haplotypes	Number of specimens
Baberspan Nature Reserve	0.5696 ± 0.1962	4	5
Bloemfontein Glen	0.0916 ± 0.0765	2	3
Bloemfontein	0.1567 ± 0.1404	2	2
Chelmsford Nature Reserve	0.0000	1	3
Ermelo	0.0913 ± 0.0821	2	3
Ghanzi	-	1	1
Kalahari Gemsbok National Park	0.0000	1	2
Karoo National Park	-	1	1
Kimberley	0.1373 ± 0.1290	2	2
Kroonstad	0.2868 ± 0.1496	3	4
Kuruman	0.0000	1	3
Pretoria	0.0000	1	6
Queenstown	0.0000	1	2
Riemvasmaak	-	1	1
Sandveld Nature Reserve	0.2814 ± 0.2034	2	2
Stellenbosch	-	1	1
Tussen-die-Rivieren Game Farm	-	1	1
Van Zylsrus	-	1	1
Victoria West	0.0000	1	2
Willem Pretorius Nature Reserve	-	1	1
Windhoek	0.0000	1	4

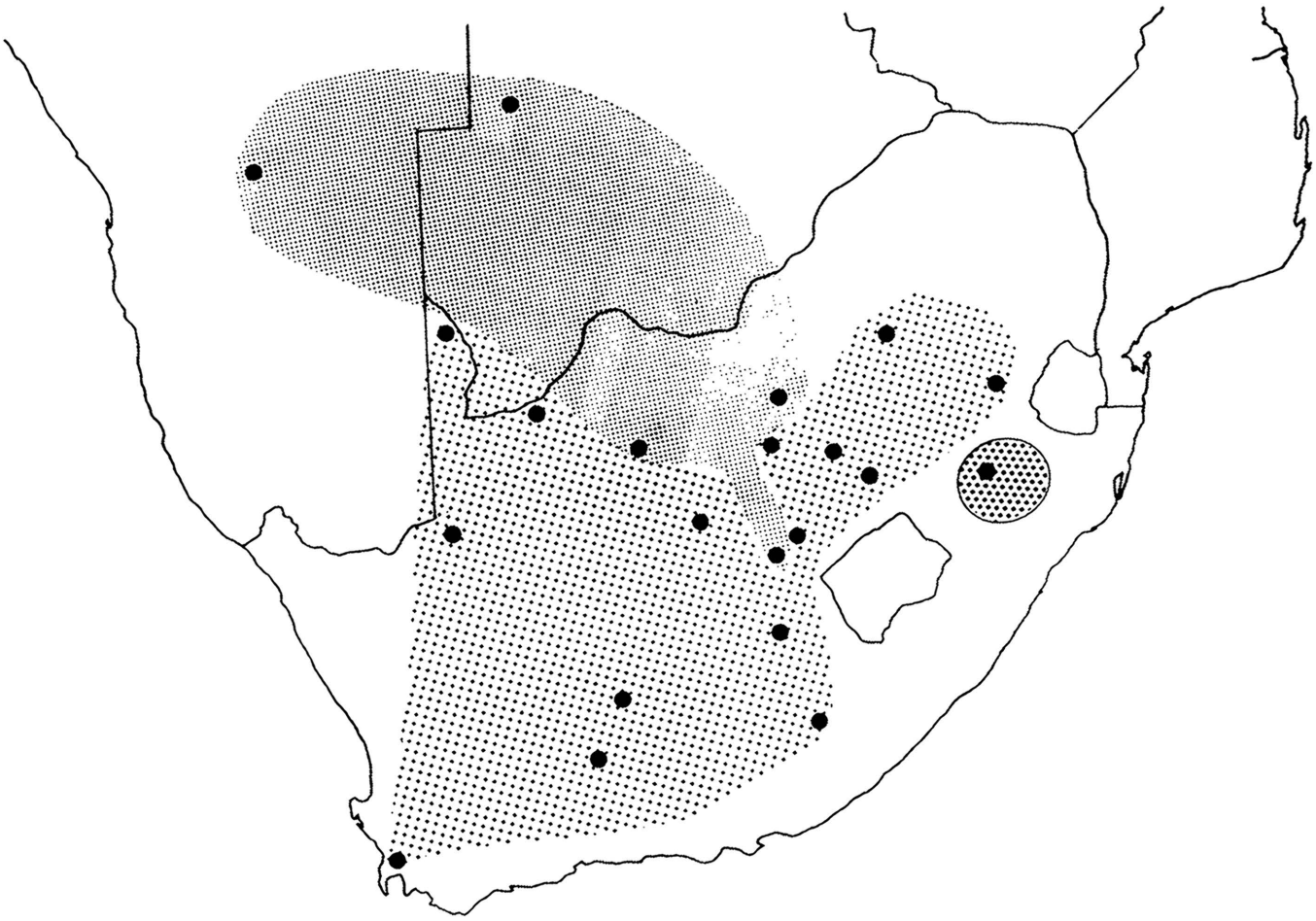


Figure 10: Geographic distribution of the mitochondrial DNA assemblages based on cluster analysis of the sample populations.

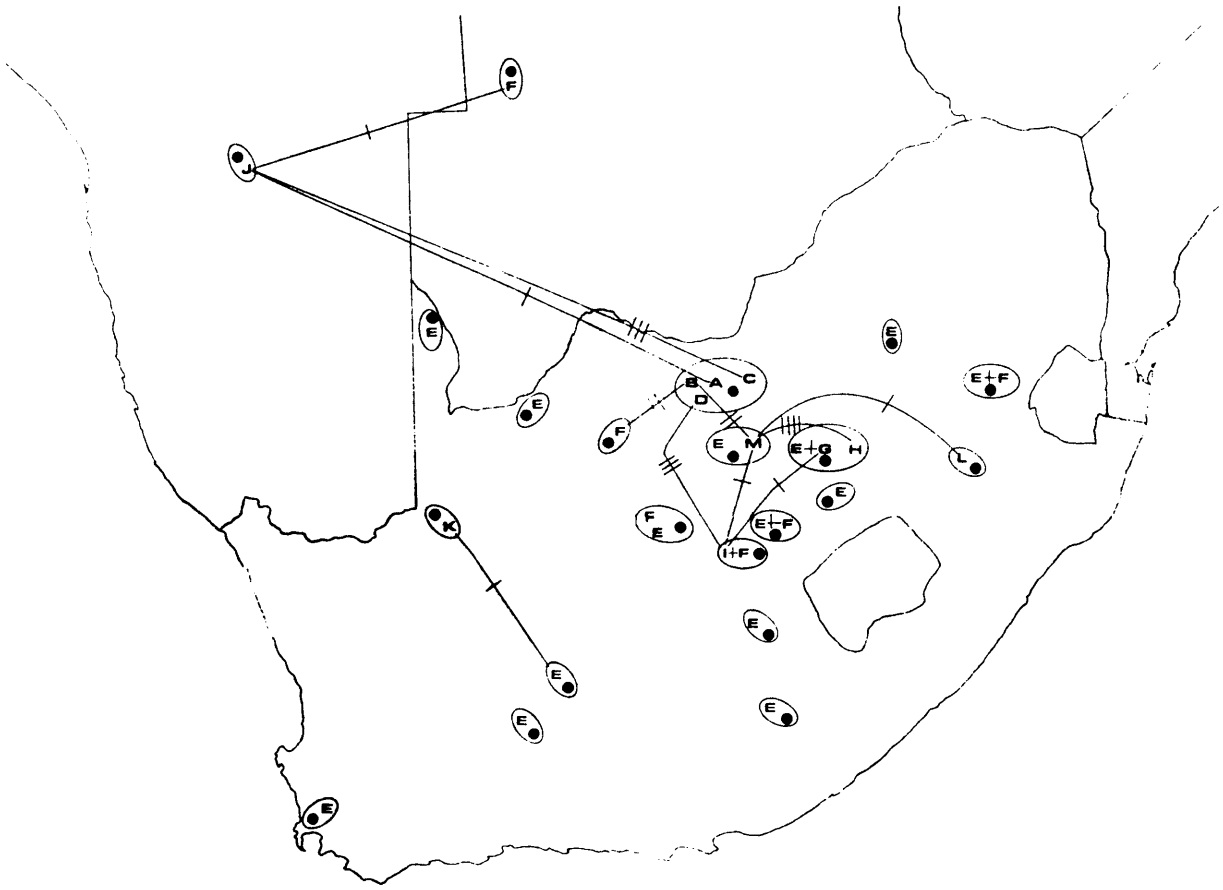


Figure 11: Parsimony generated mitochondrial phylogeny for the yellow mongoose superimposed on the southern African sources of collection. The number of mutational steps separating the maternal lineages are indicated by the cross-bars on the solid lines.

NON-CODING REGION ANALYSES

Restriction endonuclease analysis

In an attempt to improve the resolution obtained using RFLP analysis, seventeen specimens representative of all thirteen maternal RFLP lineages (see Fig.8) were chosen for restriction enzyme analysis of the major non-coding region. On PCR amplification using primers THRL1 and DLH1, these 17 specimens grouped as two distinct haplotypes, their distinction based on the presence/absence of an 81 bp insert. Four (E, G, H, and K) of the thirteen different maternal haplotypes amplified the longer fragment. RFLP analysis of the 600 bp PCR fragment using a suite of seven four-base recognition enzymes (see Table 1) revealed no restriction site differences between haplotypes, this approach therefore clearly not providing increased phylogeographic resolution. However, in an attempt to determine whether the two different length types are geographically discrete, specimens amplifying the two length types were overlaid onto a map of southern Africa but no meaningful pattern was evident (data not shown). In fact, several populations were found to contain both length types (Ermelo, Bloemfontein Glen, Sandveld Nature Reserve, Kimberley).

Sequencing

In an attempt to identify the cause of the length difference detected within the major non-coding region five specimens were sequenced. These included two that amplified the larger fragment (mitochondrial DNA haplotypes E and H, see Table 2) and three that were characterized by the shorter fragment (mitochondrial DNA haplotypes D, J and L, see Table 2). Selection was also based on their positions in the NJ tree topology (see Fig.8).

It is noteworthy that the presence/absence of this 81 bp insert was not evident when scoring mitochondrial RFLPs from sample specimens. This is probably attributable to the relatively small size of the insert - a size difference of < 1% of the average of two fragments being compared is not readily detectable (see Jin & Nei 1991).

Direct sequencing of the control region revealed the length difference to be due to an 81 bp direct repeat in the specimens amplifying the larger segment (mitochondrial DNA haplotypes E and H, nucleotide sequence positions 284 to 364; Appendix 3). No other deletions\insertions were detected among the *Cynictis* specimens studied.

In total, 482 bp on the 5' side of the non-coding region were aligned for all ingroup taxa as well as the outgroup species *C. lupis* and *C. aureus* (Appendix 3). The sequence homology between the yellow mongoose haplotypes was high (Table 6). For the parsimony analysis, positions 283 to 364 (which correspond to the insert) were excluded. Of the 401 bp aligned for all seven taxa, 90 characters were found to be variable, 43 of these were informative. However, if the outgroups were excluded, only nine variable sites, two of which represented parsimony sites, were found. Within the five yellow mongoose specimens, transitions were found to outweighed transversions 7:3 (81 bp repeat excluded).

The exhaustive search option of the PAUP computer program produced two equally parsimonious trees of 537 steps. A 50% majority rule consensus of these trees is shown in Fig.12. The tree has a CI = 1.00 and a g_1 = -2.47, indicating that the data deviate significantly from random data (Hillis & Huelsenbeck 1992).

The branch and bound parsimony bootstrap option (1000 iterations) of PAUP was used to assess the confidence of the clades recovered. The bootstrap values obtained for the different clades (Fig.12) were low, indicating that the nodes are not statistically significant at the 95% confidence level (Hillis & Bull 1993).

Table 6: Number of nucleotide changes in the major non-coding region between five different mitochondrial DNA haplotypes (below the diagonal line), and the mean distance, corrected for missing data as well as multiple hits (Jukes & Cantor 1969), above the diagonal line.

	E	H	J	D	L
E	-	0.004	0.012	0.010	0.012
H	2	-	0.010	0.007	0.010
J	5	4	-	0.012	0.015
D	4	3	5	-	0.007
L	5	4	6	3	-

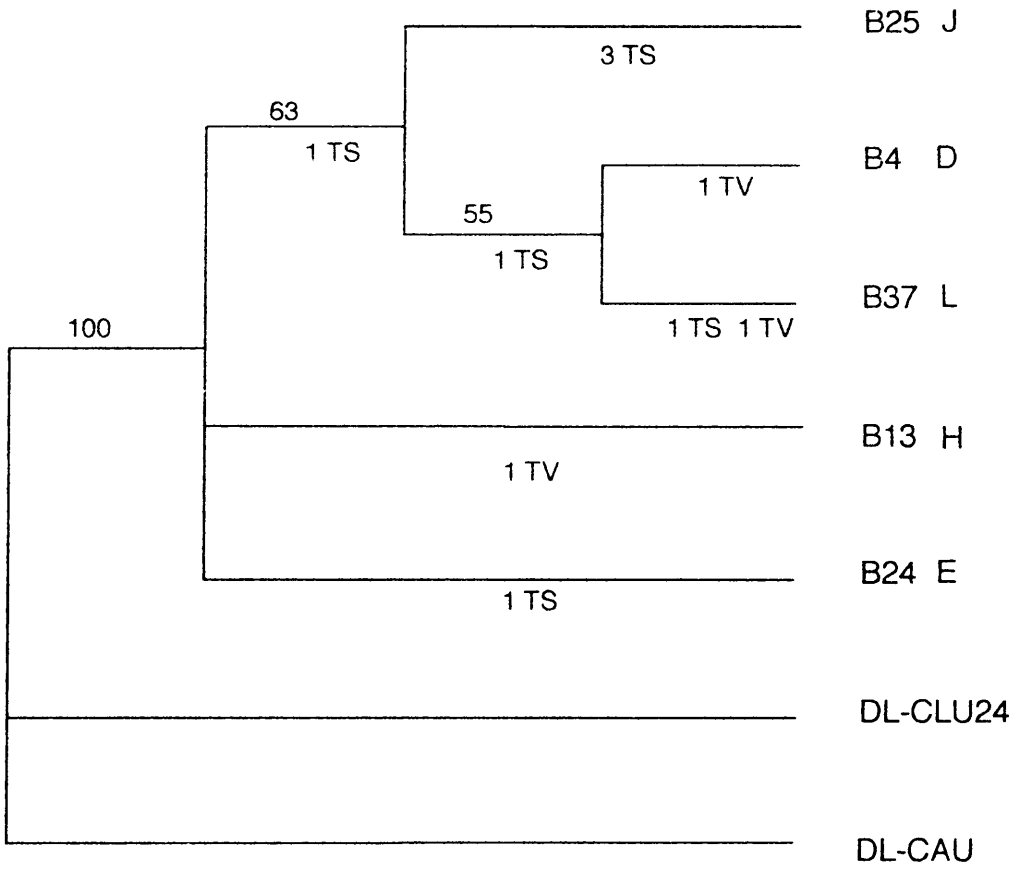


Figure 12: A 50% majority rule consensus tree based on the two most parsimonious trees generated by the exhaustive search option in PAUP. Numbers above the branches indicate bootstrap support (1000 iterations). The number of transitions (TS) and transversions (TV) occurring along the branches are indicated.

CHAPTER 4
DISCUSSION AND CONCLUSION

PHYLOGEOGRAPHIC POPULATION STRUCTURE

The mitochondrial DNA phylogeography of a species is influenced by the historical pattern of gene flow which, in turn, is a function of the extrinsic (zoogeographic) impediments to movement and the intrinsic dispersal capabilities of the species (Ball *et al.* 1988; Hayes & Harrison 1992). Two distinct aspects of the mitochondrial DNA data demonstrate the limited geographic partitioning seen in the yellow mongoose. First, genetic distances estimated in terms of base substitution per nucleotide site between the majority of haplotypes are small (Table 4), revealing a close relationship between them (Fig.7). Secondly, the most frequent maternal lineage (E) is present in populations throughout the species' distribution (Fig.11), and the high frequency, in concert with the close association between this haplotype and the majority of other haplotypes, suggest that it is ancestral or ancestral-like. The F haplotype, which is also widespread, forms the hub of the parsimony network (Fig.7). It is closely affiliated to E, and is therefore not at variance with the proposed ancestral state of the E haplotype.

Except for the E and F haplotypes which are widely distributed, most other mitochondrial DNA haplotypes are geographically localized (Fig.11). Although the majority of yellow mongoose populations are characterized by a single maternal lineage, some populations were polytypic. This is reflected in the intra-populational nucleotide diversity which varied between 0, where all specimens were characterized by a single maternal lineage, to 0.5696 ± 0.1962, calculated for the Baberspan Nature Reserve population (Table 5). The five specimens collected at this locality revealed four distinct maternal lineages which include the two most divergent matrilines encountered in the present study (C and D). This may be indicative of the antiquity and large size of the population which, in concert, may have allowed the relatively high number of mutational changes to accumulate within

the area represented by the Baberspan Nature Reserve population. Alternatively, the elevated levels of variation detected in this population can also be ascribed to its location within an ecological transition zone. The Reserve is situated in the region between the Savanna biome, which extends narrowly into the western side of South Africa (but has a broad distribution further north), and the Grassland biome which covers the central part of South Africa (Rauterford & Westfall 1986). Evidence of past migration into the Baberspan Nature Reserve population is to some extent supported by the low divergence value separating the J haplotype found in the Windhoek population and the A haplotype (Baberspan Nature Reserve population). A similar case was reported for the black-backed jackal (*C. mesomelas*; Wayne *et al.* 1990) where the two most divergent haplotypes detected in that study co-occur at the same geographic locality. Wayne and co-workers (1990) interpret this to be the result of the species' significant dispersal capabilities. Dispersion in carnivores depends primarily on physiological tolerance and body size and, secondarily, on the life history (Gittleman 1985, 1989). Little is known about dispersion in the yellow mongoose. Intuitively, however, this would be thought to be restricted because of the small body size of the species and because individuals seldom move further than the neighbouring colony (O.A.E.Rasa, pers com.).

Results from both NJ and UPGMA reveal three major mitochondrial DNA assemblages (Fig.10) which correspond loosely to the recognized morphometric subspecies (Taylor 1990; Taylor & Meester 1993). The lack of strict congruence between the mitochondrially defined clades and the morphotypes is not unexpected since subspecies are generally not geographically sharply defined. This is particularly evident between *C. p. penicillata* and *C. p. bradfieldi*. Additionally, the mitochondrial DNA data do not distinguish between the two northern morphometric subspecies (*C. p. coombsii* and *C. p. bradfieldi*) although this may be an artefact of sampling. The geographic coverage of the two northern morphometric subspecies are represented by few specimens in this study and further

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sampling may reveal the presence of additional maternal lineages. It seems likely, therefore, that the morphological differences between yellow mongoose populations occurred against a backdrop of little or no molecular separation.

Comparative studies have shown that morphological change and molecular divergence are quite independent, responding to different evolutionary pressures and following different evolutionary rules (Wilson, Sarich & Maxson 1974 in Hillis & Moritz 1990). Ball and co-workers (1988) suggest two possible explanations to account for this phenomenon. First, that morphological differences are primarily ecophenotypic in nature and not based entirely on genetic differences. This, however, does not seem to be the case for the yellow mongoose. Multivariate analysis of geographic variation by Taylor (1990) and Taylor and Meester (1993) revealed the presence of the discrete morphotypes within the species. The morphological differences detected are, therefore, not clinal and, by extension, environmentally induced. Secondly, that genes responsible for morphological differences evolve so rapidly that geographic differentiation has arisen over a time scale too short to have been readily detected by mitochondrial DNA (Ball *et al.* 1988). If this latter suggestion holds, it would seem to suggest that the yellow mongoose is a demographically young species whose modern distributional range represents a recent, rapid colonization initiated by relatively few individuals from a refugial area. Fragmentation and colonization could have been prompted by environmental change for example, generally wetter conditions prevailed in the Kalahari between 32 000 and 22 000 years ago and the last major wet cycle in the Karoo-Namaqualian ecozone was between 30 000 and 13 000 years ago (Klein 1984). Under more humid conditions, semi-arid habitats are thought to have contracted and mesic habitats expanded, a sequence of events which would have led to fragmentation of the yellow mongoose's range. The onset of the subsequent dry cycle would have caused semi-arid habitats to expand, thus making suitable habitat available to the yellow mongoose, invariably leading to the rapid range expansion of the species.

Cluster analysis revealed the Natal population (Chelmsford Nature Reserve) to be the most divergent being separated by 0.58% sequence divergence from the other populations (Fig.9). Genetic differences between the Natal assemblage and the remaining yellow mongoose clusters are comparable to differences detected in another carnivore species, the wild dog (*Lycaon pictus*). In this species, two major wild dog mitochondrial clades (east and southern African) were detected which are separated by a sequence divergence of between 0.7 and 0.9% (Girman *et al.* 1993). These data led these authors to argue that these groupings represent distinct subspecies. While the *C. p. natalensis* sequence divergence values are not that dissimilar from those detected for the wild dog, there are obvious dangers in extrapolation from one species to another. Nonetheless, given these data, and the fact that the mitochondrial DNA delineation of the Natal assemblage is congruent with the morphometric separation of this subspecies, there appears to be good support for the recognition of *C. p. natalensis* as a taxonomic unit, the prior name for which is *C. p. penicillata*. When viewed from a topographical perspective, it is striking that the Natal population appears, for all purposes, to be a geographic isolate. The Drakensberg mountain range, which forms part of the Great Escarpment, presumably acts as a barrier to gene flow between yellow mongoose populations east of the escarpment and those situated westwards of this barrier.

MAJOR NON-CODING REGION

Mignotte *et al.* (1990) reported that although the central-conserved sequence in the control region appears to be one of the mitochondrial DNA's most conserved regions, other parts of the non-coding region represent the least conserved sequences of the molecule. Since mitochondrial DNA RFLP studies survey restriction site variation distributed around the whole mitochondrial DNA molecule, most of which codes and is thus more conserved than the control region (Anderson *et al.* 1981, Anderson, De Bruijn, Coulson, Eperon, Sanger & Young 1982), the close sequence correspondence between the five yellow mongoose specimens utilized for this aspect of the investigation, was somewhat unexpected. The species is not, however, unique in this respect.

Comparisons between cetacean species (Southern, Southern & Dizon 1988; Árnason, Gullberg & Widegren 1991) show similar levels of nucleotide substitution and difference between cetacean control regions approximated those detected in the rest of the mitochondrial DNA molecule. Comparable findings were reported for the rat and mouse where the rate of base substitutions in the control region, as judged by mitochondrial DNA RFLPs (Brown, Gadaleta, Pepe, Saccone & Sbisa 1986), is similar to, and even slightly less, than that of protein coding genes. The variation in the yellow mongoose's control region is lower than that detected for the complete mitochondrial molecule (see Table 4 and 6). These data and those published for species as divergent as cetaceans and rodents question the assumption of a universally high turnover rate in the mitochondrial DNA control region of mammals.

A possible explanation to account for the relatively low mutation rate observed for the control region in the yellow mongoose, cetaceans and rodents may be related to a mutational bias. In vertebrates there is a strong transition:transversion (TS:TV) bias in both protein-coding and non-coding regions, where a ratio as high as 20:1 has been reported for the cytochrome b gene of Australian songbirds (*Pomatostomus*; Edwards & Wilson 1990). In the brook charr (*Salvelinus fontinalis*; Bernatchez & Danzmann 1993), this ratio was 8:3 which led these authors to speculate that transitional mutations may be more restricted (or even selected against) in the control region, thus leading to the decrease in the detectable mutation rate. The TS:TV ratio detected for the brook charr is not dissimilar to that of the yellow mongoose, where a value of 7:3 (81 bp repeat excluded) was found. Since the evolutionary pressure on the non-coding region appears to take place at the level of the secondary structure rather than at the level of nucleotide sequences (Mignotte *et al.* 1990), Bernatchez and Danzmann (1993) proposed that the lower occurrence of transitions is related to the structural or functional needs of maintaining a non-random nucleotide composition in the control region. Given that any nucleotide can change from its original to three remaining states (not taking back mutations into account), two of these mutations would be transversions and one a transition. If there is selective pressure on a non-random nucleotide distribution in the

major non-coding region, it could be anticipated that mutations which alter the AT:GC content of the control region would be selected against since these are more likely to affect secondary structure and, by implication, the replication of the mitochondrial DNA molecule. While this could account for the low transition:transversion ratio in the yellow mongoose, too few mutations were observed to rigorously test this hypothesis.

PCR amplification of the 5' end of the control region revealed the presence of a length polymorphism within the yellow mongoose. No phylogeographic signal is contained in this variant since maternal RFLP lineages that are characterized by the larger PCR amplified product (E, G, H and K) co-occur with haplotypes that amplify the shorter PCR fragment (Ermelo, Kimberley, Sandveld Nature Reserve, Bloemfontein Glen). Direct sequencing of the segment of the control region targeted by the primers used in this study showed the polymorphism to be due to an 81 bp direct repeat sequence. Clearly, a similar duplication or deletion event occurring independently in two evolutionary lineages is unlikely. The most parsimonious explanation would be that the length polymorphism originated once in an ancestral lineage, and that all the descendants of the lineage would therefore carry the mutation. This, however, does not appear to be the case in the yellow mongoose. Only three of the four mitochondrial DNA RFLP haplotypes that amplify the larger fragment (E, K & G) occur in the same cluster as reflected by the NJ tree (Fig.8). The fourth RFLP haplotype (H), falls outside of this assemblage, grouping together with haplotypes that are characterized by the shorter PCR fragment, suggesting two independent origins for the length polymorphism.

In contrast, however, parsimony analysis (Fig.12) suggests that the 81 bp repeat (Haplotypes E and H) is a plesiomorphic character that was subsequently lost in the lineage that led to the J, D and L mitochondrial DNA RFLP haplotypes. A single loss of the 81 bp insert would be more parsimonious than two independent gains (as suggested by the NJ tree; Fig.8). However, given the low number of informative characters available for the PAUP analysis, the origin of the length polymorphism detected within *Cynictis* remains unresolved. Parsimony further revealed few mutational changes along the

internal branches (short internal branch lengths; Fig.12), with the majority of changes accumulating at the tips. This provides additional evidence for a rapid colonization event in the species' evolutionary past, followed by a relatively stationary period.

RABIES

The viverrid-type rabies, with the yellow mongoose as the chief disseminator within the southern African subregion (Meredith 1982), has a fairly localized geographic occurrence, given the wider distributional range of *Cynictis* (see Figs.2 and 3). Two possible explanations can account for this. The first may be density related with the viverrid-type rabies coinciding loosely with the area of greatest yellow mongoose density. The sandveld regions of the central and northwestern Orange Free State and Northwest Provinces have conventionally been regarded as areas of high yellow mongoose density (Snyman 1940). The rabies virus is only present in the saliva of infected animals 7 days prior to death (Chaparro & Esterhuisen 1993); saliva is the usual route of transmission and the species' communal, burrow-dwelling lifestyle is probably conducive to its spread. In contrast, the yellow mongooses is reported (Zumpt 1976; Barnard 1979) to be characterized by a more solitary life style in the northern reaches of its distribution (and hence lower population densities), thus reducing the probability of spreading of the virus.

A second explanation to account for the restricted occurrence of the viverrid-type rabies is that a genetic factor(s) preferentially underpin the susceptibility of local demes to the disease. In turn, these foci are maintained by restricted gene flow between populations whose presence could be reflected in a non-causal manner by suitable genetic markers. Although speculative, the pattern of association between the mitochondrial DNA data and the morphometrically defined *C. p. penicillata* and, in turn, their congruence with the geographic limits of viverrid-type rabies, may be regarded as providing some support for a genetic basis to the distribution of the disease. However, in the absence of more data, a genetic explanation to account for the unique pattern of viverrid rabies must remain equivocal.

SUMMARY

The phylogeographic population structure was determined for the yellow mongoose (*C. penicillata*), a small carnivore species endemic to southern Africa. Mitochondrial DNA restriction fragment length polymorphism (RFLP) analysis of 50 specimens revealed a weak genetic structure with little phylogenetic partitioning corresponding to geographic barriers. It is postulated that the close association between matrilines is attributable to a bottleneck in the recent evolutionary past of the species. Cluster analysis, based on the yellow mongoose populations sampled, revealed three mitochondrial groups. Although these correspond loosely with the recently proposed morphometric subspecies, *C. p. penicillata, C. p. bradfieldi, C. p. coombsii* and *C. p. natalensis* (Taylor 1990), the central and northern clusters are separated by low sequence divergence values. The Natal cluster was found to be the most divergent and the mitochondrial data, in conjunction with morphometric data, support subspecific status for this group. Additional samples might strengthen this argument, as both the mitochondrial and morphological evidence are based on small sample sizes.

To increase the level of resolution obtained with RFLP analysis, a portion of the major non-coding region was chosen for further investigation. Four-base recognition restriction enzyme digestions of approximately 600 bp, amplified by the polymerase chain reaction (PCR), failed to reveal polymorphic cut sites between the 17 representative specimens. The study did, however, define two control region haplotypes which differ in size through the presence of an 81 bp repeat, with four of the RFLP composite haplotypes consistently amplifying a larger fragment. To clarify the length difference, PCR products from five specimens, two that amplified the larger and three that amplified the shorter PCR fragment, were sequenced.

The restricted occurrence of the viverrid-type rabies within the extensive yellow mongoose distribution raises the question of possible genetic factor(s) affecting the susceptibility of local demes to the disease. The congruence evident between the viverrid-type rabies

region, the central mitochondrial DNA cluster and the morphometrically defined *C. p. penicillata* may provide some support for the hypothesis of a genetic basis to the distribution of rabies in southern Africa.

OPSOMMING

Die filogeografiese bevolkingsstruktuur is bepaal vir die witkwasmuishond (*C. penicillata*), 'n klein karnivoor endemies tot suidelike Afrika. Mitochondriale DNA restriksie fragment lengte polimorfisme (RFLP) analise van 50 eksemplare het min mitochondriale DNA diversiteit aangetoon, met min of geen filogenetiese skeiding wat ooreenstem met geografiese versperrings. Die noue assosiasie tussen die materne lyne word toegeskryf aan 'n bottelnek in die onlangse evolusionêre verlede van die spesie. Groeperingsanalise, gebaseer op die witkwasmuishond bevolkings in die huidige studie het drie mitochondriale groepe uitgelig. Alhoewel hierdie groepe naastenby ooreenstem met die huidiglike voorgestelde morfometriese subspesies, *C. p. penicillata, C. p. bradfieldi, C. p. coombsii* en *C. p. natalensis* (Taylor 1990), word die sentrale en noordelike groepe van mekaar geskei deur 'n lae nukleotied-opeenvolging-diversiteitswaardes. Die Natal groep was die mees divergente, en die mitochondriale data, sowel as die morfometriese data, ondersteun subspesie status vir hierdie groep. Addisionele eksemplare mag hierdie argument bevorder aangesien beide die mitochondriale en die morfometriese bewyse gebaseer is op 'n beperkte aantal eksemplare.

'n Deel van die nie-koderende gebied is gekies vir verdere ondersoek om die resolusie wat verkry is met die RFLP analise, te verhoog. Vier-basis herkennings restriksie ensiem analises van ongeveer 600 bp, geamplifiseer deur die polimerase kettingreaksie (PCR), het geen polimorfismes tussen die 17 verteenwoordigende eksemplare aangetoon nie. Die studie het egter twee haplotipes gedefinieer wat verskil van mekaar ten opsigte van die herhaling van 81 bp, vier van die 17 RFLP haplotipes amplifiseer die langer fragment. Om die verskil in lengte te verklaar, is die amplifikasie produkte van vyf eksemplare, twee wat die langer fragment en drie wat die korter PCR fragment amplifiseer, se nukleotied-opeenvolgings-volgorde bepaal.

Die beperkte verspreiding van die muishond tipe hondsdolheid, binne die wyer geografiese verspreiding van die witkwasmuishond, laat die vraag ontstaan oor die moontlikheid van 'n genetiese faktor(e) wat die ontvanklikheid van die spesie beinvloed vir hondsdolheid. Die sigbare ooreenkoms tussen die muishond tipe hondsdolheid en die sentrale mitochondriale DNA groep, asook die morfometriese gedefinieerde *C. p. penicillata,* mag ondersteuning verleen aan die hipotese dat daar 'n genetiese basis bestaan vir die verspereiding van hondsdolheid in Suid-Afrika.

REFERENCES

ALLEN, G.M. 1939. A check list of African mammals. *Bulletin of the Museum of Comparative Zoology at Harvard College.* 83:1-763.

ANDERSON, S., BANKIER, A.T., BARRELL, B.G., DE BRUIJN, M.H.L.,COULSON, A.R., DROUIN, J., EPERON, I.C., NIERLICH, D.P., ROE, B.A., SANGER, F., SCHREIER, P.H., SMITH, A.J.H., STADEN, R. & YOUNG, I.G. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290:457-465.

ANDERSON, S., DE BRUIJN, M.H.L., COULSON, A.R., EPERON, I.C., SANGER, F. & YOUNG, I.G. 1982. Complete sequence of bovine mitochondrial DNA, conserved features of the mammalian genome. *J. Mol. Biol.* 156: 683-717.

AMOS, B. & HOELZEL, A.R. 1991. Longterm preservation of whale skin for DNA analysis. *Rep. Int. Whaling Comm.* 13: 99-104.

ÁRNASON, Ú., GULLBERG, A. & WIDEGREN, B. 1991. The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus. J. Mol. Evol.* 33:556-568.

ÁRNASON, Ú. & JOHNSSON, E. 1992. The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina. J. Mol. Evol.* 34:493-505.

AVISE, J.C. 1991. Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. *Ann. Rev. Genet.* 25:45-69.

AVISE, J.C., ARNOLD, J., BALL, R.M., BERMINGHAM, E., LAMB, T., NEIGEL, J.E., REEB, C.A. & SAUNDERS, N.C. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Ann. Rev. Ecol. Syst.* 18:489-522.

AVISE, J.C., BOWEN, B.W. & LAMB, T. 1989. DNA fingerprints from hypervariable mitochondrial genotypes. *Mol. Biol. Evol.* 6:258-269.

AVISE, J.C., BOWEN, B.W., LAMB, T., MEYLAN, A.B. & BERMINGHAM, E. 1992. Mitochondrial DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the Testudines. *Mol. Biol. Evol.* 9:457-473.

AVISE, J.C. & LANSMAN, R.A. 1983. Polymorphism of mitochondrial DNA in populations of higher animals. In: *Evolution of Genes and Proteins*, (eds.) M.Nei & R.K. Koehn, pp. 147-164. Sinauer, Massachusetts.

AVISE, J.C., LANSMAN, R.A. & SHADE, R.O. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. *Genetics* 92:279-295.

AVISE, J.C., NEIGEL, J.E. & ARNOLD, J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *J. Mol. Evol.* 20:99-105.

BALL, R.M., FREEMAN, S., JAMES, F.C., BERMINGHAM, E. & AVISE, J.C. 1988. Phylogeographic population structure of red-winged blackbirds assessed by mitochondrial DNA. *Proc. Natl. Acad. Sci. USA.* 85: 1558-1562.

BARNARD, B.J.H. 1979. The role played by wildlife in the epizootology of rabies in South Africa and South-West Africa. *Onderstepoort J. Vet. Res.* 46: 155-163.

BARTON, N. & JONES, J.S. 1983. Mitochondrial DNA: New clues about evolution. *Nature* 306:317-318.

BERNATCHEZ, L. & DANZMANN, R.G. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Mol. Biol. Evol.* 10: 1002-1014.

BIBB, M.J., VAN ETTEN, R.A., WRIGHT, C.T., WALBERG, M.W. & CLAYTON, D.A. 1981. Sequence and gene organization of mouse mitochondrial DNA. *Cell* 26:167-180.

BROWN,G.G., GADALETA, G., PEPE, G., SACCONE, C. & SBISA, E. 1986. Structural conservation and variation in the D-loop containing region of vertebrate mitochondrial DNA. *J. Mol. Evol.* 192:503-511.

BROWN, W.M. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. *Proc. Natl. Acad. Sci. USA.* 77: 3605-3609.

BROWN, W.M. 1983. Evolution of animal mitochondrial DNA. In: *Evolution of genes and proteins*, (eds.) M. Nei & R.K. Koehn, pp. 62-88. Sinauer, Massachusetts.

BROWN, W.M., GEORGE, M. & WILSON, A.C. 1979. Rapid evolution of mitochondrial DNA. *Proc. Nat. Acad. Sci. USA.* 76: 1967-1971.

BROWN, W.M., PRAGER, E.M., WANG, A. & WILSON, A.C. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J. Mol. Evol.* 18:225-239.

BUGGE, J. 1978. The cephalic arterial system in carnivores, with special reference to the systematic classification. *Acta Anat.* 101:45-61.

BUROKER, N.E., BROWN, J.R., GILBERT, T.A., O'HARA, P.J., BECKENBACH, A.T., THOMAS, W.K. & SMITH, M.J. 1990. Length heteroplasmy of sturgeon mitochondrial DNA: An illegitimate elongation model. *Genetics* 124:157-163.

CANN, R.L. & WILSON, A.C. 1983. Length mutations in human mitochondrial DNA. *Genetics* 104:699-711.

CARROLL, R.L. 1988. *Vertebrate Paleontology and Evolution.* pp. 478-483. W.H. Freeman and Company, San Francisco.

CHAPARRO, F. & ESTERHUYSEN, J.J. 1993. The role of the yellow mongoose (*C.penicillata*) in the epidemiology of rabies in South Africa - preliminary results. *Onderstepoort J. Vet. Res.* 60:373-377.

CUMMING, D.H.M. 1982. A case history of the spread of rabies in an African country. *S.A. J. Science* 78:443-447.

CUVIER, G. 1829. *Le regne animal.* Second edition.

DEBLASE, A.F. & MARTIN, R.E. 1981. *A manual of mammalogy with keys to families of the world.* W.M.C.Brown Co. Dubuque.

DEBRY, R.W. 1992. The consistency of several phylogeny-inference methods under varying evolutionary rates. *Mol. Biol. Evol.* 9:537-551.

DESJARDINS, P. & MORAIS, R. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *J. Mol. Biol.* 212:599-634.

EDWARDS, S.V. & WILSON, A.C. 1990. Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). *Genetics* 126:695-711.

EFSTRATIADIS, A., POSAKONY, J.W., MANIATIS,T., LAWN, R.W., O'CONNELL, C., SPRITZ, A., DERIEL, J.K., FORGET, B.G., WEISSMAN, S.M., SLIGHTOM, L., BLECHI, A.E., SMITHIES, O., BARALLE, F.E., SHOULDERS, C.C. & PROUDFOOT, N.J. 1980. The structure and evolution of the human β-globin gene family. *Cell* 21:653-668.

ELLERMAN, J.R., MORRISON-SCOTT, T.C.S., & HAYMAN, R.W. 1953. *Southern African mammals 1758 to 1951; a reclassification.* British Museum (NH), London.

FEINBERG, A.P. & VOGELSTEIN, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.

FELSENSTEIN, J. 1993. PHYLIP: phylogenetic information package, version 3.5c.

FREDGA, K. 1972. Comparative chromosome studies in mongooses (Carnivora, Viverridae). I. Idiograms of 12 species and karyotype evolution in Herpestinae. *Hereditas* 71:1-74.

GIRMAN, D.J., KAT, P.W., MILLS, M.G.L., GINSBERG, J.R., BORNER, M., WILSON, V., FANSHAWE, J.H., FITZGIBBON, C., LAU, L.M. & WAYNE, R.K. 1993. Molecular genetic and morphological analysis of the African wild dog (*Lycaon pictus*). *J. Hered.* 84: 450-459.

GITTLEMAN, J.L. 1985. Carnivore body size: Ecological and taxonomic correlates. *Oecologia* 67:540-554.

GITTLEMAN, J.L. 1989. Carnivore group living: Comparative trends. In: *Carnivore behaviour, ecology and evolution,* (ed.) J. L. Gittleman. Cornell University Press, New York.

GRAVEN, L., PASSARINO, G., SEMINO, O., BOURSOT, P., SANTACHIARA-BENERECETTI, S., LANGANEY, A. & EXCOFFIER, L. 1995. Evolutionary correlation between control region sequence and restriction polymorphisms in the mitochondrial genome of a large Senegalese Mandenka sample. *Mol. Biol. Evol.* 12: 334-345.

GYLLENSTEN, U., WHARTON, D., JOSEFSSON, A. & WILSON, A.C. 1991. Paternal inheritance of mitochondrial DNA in mice. *Nature* 352:255-257.

HALE, L.R. & SINGH, R.S. 1991. A comprehensive study of genetic variation in natural populations of Drosophila melanogaster. IV. Mitochondrial DNA variation and the role of history vs. selection in the genetic structure of geographic populations. *Genetics* 129:103-117.

HARLEY, E.H. 1994. DAPSA: DNA and protein sequence analysis, version 2.9.

HARRISON, R.G. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *TREE.* 4: 6-11.

HARRISON, R.G., RAND, D.M. & WHEELER, W.C. 1985. Mitochondrial DNA size variation within individual crickets. *Science* 228:1446-1448.

HAYASAKA, K., ISHIDA, T. & HORAI, S. 1991. Heteroplasmy and polymorphism in the major noncoding region of mitochondrial DNA in Japanese monkeys: Association with tandemly repeated sequences. *Mol. Biol. Evol.* 8:399-415.

HAYES, J.P. & HARRISON, R.G. 1992. Variation in mitochondrial DNA and the biogeographic history of woodrats (*Neotoma*) of the eastern United States. *Syst. Biol.* 41: 331-344.

HENDEY, Q.B. 1974. Faunal dating of the late Cenozoic of Southern Africa, with special reference to the Carnivora. *Quat. Res.* 4:149-161.

HILLIS, D.M. & BULL, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182-192.

HILLIS, D.M. & MORITZ, C. 1990. *Molecular systematics*, (eds.) D.M. Hillis & C. Moritz. Sinauer, Massachusetts.

HOELZEL, A.R. 1993. Evolution by DNA turnover in the control region of vertebrate mitochondrial DNA. *Current opinion in Genetics and Development.* 3: 891-895.

HOELZEL, A.R. & DOVER, G.A. 1991. Mitochondrial D-loop DNA variation within and between populations of the mink whale (*Balaenoptera acutorostrata*). In: *Genetic ecology of whales and dolphins. Report of the International Whaling Commission, special issue 13,* (ed.) A.R. Hoelzel, pp. 171-181.

HONACKI, J.H., KINMAN, K.E. & KOEPPL, J.W. 1982. *Mammal Species of the World: A Taxonomic and Geographic Reference.* Lawrence, Kan.: Association of Systematics Collections.

HOWELL, P.G. 1982. Rabies and rabies-like viruses. *S.A. J. Science* 78:409-411.

HUNT, R.M. 1987. Evolution of the Aeluroid Carnivora: Significance of auditory structure in the nimravid cat *Dinictis. Am. Mus. Nov.* 2886:1-74.

JIN, L. & NEI, M. 1991. Relative efficiencies of the maximum- parsimony and distance-matrix methods of phylogeny construction for restriction data. *Mol. Biol. Evol.* 8:356-365.

JUKES, T.H. & CANTOR, C.R. 1969. *Evolution of protein molecules.* H.N. Munro. Academic Press, New York.

KING, A.A., MEREDITH, C.D. & THOMSON, G.R. 1995. The biology of southern African lyssavirus variants. *Cur. Topics Micro. Immun.* 187:267-295.

KLEIN, R.G. 1984. *Southern African prehistory and paleoenvironments.* A.A. Balkema. Rotterdam, Boston.

LANSMAN, R.A., AVISE, J.C. & HUETTEL, M.D. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. *Proc. Natl. Acad. Sci. USA.* 80: 1969-1970.

LANSMAN, R.A., SHADE, R.O., SHAPIRA, J.F. & AVISE, J.C. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequences relatedness in natural populations. *J. Mol. Evol.* 17: 214-226.

LEVINSON, G. & GUTMAN, G.A. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* 4: 203-221.

LIDICKER, W.Z. 1962. The nature of subspecies boundaries in a desert rodent and its implications for subspecies taxonomy. *Syst. Zool.* 11: 160-171.

LUNDHOLM, B.G. 1955. A taxonomic study of *Cynictis penicillata* (G. Cuvier). *Ann. Trans. Mus.* 22: 305-320.

LYNCH, C.D. 1975. The distribution of mammals in the Orange Free State, South Africa. *Nav. Nas. Mus.* 3:109-139.

LYNCH, C.D. 1980. Ecology of the suricate, *Suricata suricatta* and yellow mongoose, *Cynictis penicillata*, with special reference to their reproduction. *Mem. Nas. Mus. Bloemfontein* 14: 1-145.

MANIATIS, T., FRITSCH, E.F. & SAMBROOK, J. 1982. *Molecular cloning: a laboratory manual.* Cold Spring Harbor Laboratory, U.S.A.

MARTIN, L.D. 1989. Fossil history of the terrestrial Carnivora. In: *Carnivore Behaviour, Ecology and Evolution,* (ed.) J.L. Gittleman. Cornell University Press.

MARTIN, A.P., NAYLOR, G.J.P. & PALUMBI, S.R. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* 357:153-155.

MASON, P.J. & WILLIAMS, J.G. 1985. Hybridization in the analysis of recombinant DNA. In: *Nucleic Acid Hybridization,* (eds.) B.D. Hames & S.J. Higgins, pp. 113-137. IRL Press, Oxford.

MAYR, E. & ASHLOCK, P.D. 1991. *Principles of Systematic Zoology.* McGraw-Hill, Inc., New York.

MEESTER, J.A.J., RAUTENBACH, I.L., DIPPENAAR, N.J. & BAKER, C.M. 1986. *Classification of Southern African Mammals.* Transvaal Museum, Pretoria.

MERCURE, A., RALLS, K., KOEPFLI, K.P. & WAYNE, R.K. 1993. Genetic subdivision among small canids: Mitochondrial DNA differentiation of swift, kit, and arctic foxes. *Evolution* 47:1313-1328.

MEREDITH, C.D. 1982. Wildlife rabies: Past and present in South Africa. *S.A.J. Science* 78:411-415.

MICHAELIS, B. 1972. Die Schleichkatzen (Viverriden) Afrikas. *Säugetierkundl. Mitt.* 20:1-97.

MIGNOTTE, F., GUERIDE, M., CHAMPAGNE, A. & MOUNOLOU, J. 1990. Direct repeats in the non-coding region of rabbit mitochondrial DNA: Involvement in the generation of intra- and inter-individual heterogeneity. *J. Biochem.* 194:561-571.

MONFORTE, A., BARRIO, E. & LATORRE, A. 1993. Characterization of the length polymorphism in the A+T-rich region of the *Drosophila obscura* species group. *J. Mol. Evol.* 36:214-223.

MONNEROT, M., MOUNOLOU, J. & SOLIGNAC, M. 1984. Intra-individual length heterogeneity of *Rana esculenta* mitochondrial DNA. *Biol. Cell.* 52:213-218.

MORITZ, C. & BROWN, W.M. 1987. Tandem duplications in animal mitochondrial DNAs: variation in incidence and gene content among lizards. *Proc. Natl. Acad. Sci. USA.* 84: 7183-7187.

MORITZ, C., DOWLING, T.E. & BROWN, W.M. 1987. Evolution of animal mitochondrial DNA: Biology and systematics. *Ann. Rev. Ecol. Syst.* 18:269-292.

MULLIS, K.B. & FALOONA, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Meth. Enzymol.* 15:33-350.

NEI, M. 1987. *Molecular Evolutionary Genetics.* Columbia University Press, New York.

NEI, M. & LI, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA.* 76:5269-5273.

NEI, M. & MILLER, J.C. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics* 125:873-879.

NEI, M. & TAJIMA, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97:145-163.

O'BRIEN, S.J. & EVERMANN, J.F. 1988. Interactive influence of infectious disease and genetic diversity of natural populations. *TREE.* 3: 254-259.

O'BRIEN, S.J, ROELKE, M.E., YUHKI, N., RICHARDS, K.W., JOHNSON, W.E., FRANKLIN, W.L., ANDERSON, A.E., BASS JR., O.L. BELDEN, R.C. & MARTENSON, J.S. 1990. Genetic introgression within the florida panther *Felis concolor coryi. National Geographic Research* 6:485-494.

OGILBY, W. 1833. *Proceedings of the Zoological Society, London.* 49.

PRINGLE, J.A. 1977. The distribution of mammals in Natal. Part 2: Carnivora. *Ann. Natal Mus.* 23: 93-115.

RADINSKY, L. 1975. Viverrid neuroanatomy: Phylogenetic and behavioral implications. *J. Mamm.* 6:130-150.

RAND, D.M. 1993. Endotherms, ectotherms and mitochondrial genome-size variation. *J. Mol. Evol.* 37:281-295.

RAND, D.M. & HARRISON, R.G. 1986. Mitochondrial DNA transmission genetics in crickets. *Genetics* 114:955-970.

RASA, O.A.E., WENHOLD, B.A., HOWARD, P., MARAIS, A. & PALLETT, J. 1992. Reproduction in the yellow mongoose revisited. *S. A. J. Zool.* 27: 192-195.

RAUTERFORD, M.C. & WESTFALL, R.H. 1986. Biomes of southern Africa - An objective characterization. *Memm. Bot. Soc. S.A.* 54: 1-98.

ROBERTS, A. 1951. *The Mammals of South Africa*. Central News Agency, Cape Town.

ROSEVEAR, D.R. 1974. *The carnivores of West Africa*. British Museum (Natural History), London.

ROWE-ROWE, D.T. 1992. *The carnivores of Natal*. Natal Parks Board, Pietermaritzburg.

SAITOU, N. & NEI, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406-425.

SANGER, F., NICKLEN, S. & COULSON, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA*. 74:5463-5467.

SHORTRIDGE, G.C. 1934. *The mammals of South West Africa*. William Heinemann Ltd., London.

SKEAD, C.J. 1980. *Historical mammal incidence in the Cape Province*. 1:82.

SKINNER, J.D. & SMITHERS, R.H.N. 1990. *The Mammals of the Southern African subregion*. University of Pretoria, Pretoria.

SMITH, G.P. 1976. Evolution of repeated DNA sequences by unequal crossing-over. *Science* 191: 528-535.

SMITHERS, R.H.N. 1983. *The mammals of the Southern African subregion*. University of Pretoria.

SMITHERS, R.H.N. & WILSON, V.J. 1979. *Check list and atlas of the mammals of Zimbabwe Rhodesia*. Memoirs of the National Museum and Monuments of Zimbabwe Rhodesia.

SNEATH, P.H.A. & SOKAL, R.R. 1973. *Numerical Taxonomy*. W.H. Freeman, San Francisco.

SNYMAN, P.S. 1940. *The study and control of the vectors of rabies in South Africa*. D.Sc thesis. Onderstepoort, Pretoria, South Africa.

SOLIGNAC, M., MONNEROT, M. & MOUNOLOU, J. 1986. Mitochondrial DNA evolution in the *melanogaster* species subgroup of *Drosophila*. *J. Mol. Evol.* 23:31-40.

SOUTHERN, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Evol.* 98:503-517.

SOUTHERN, Š.O., SOUTHERN, P.J. & DIZON, A.E. 1988. Molecular characterization of a cloned dolphin mitochondrial genome. *J. Mol. Evol.* 28:32-42.

STEWART, D.T. & BAKER, A.J. 1994. Patterns of sequence variation in the mitochondrial D-loop region of shrews. *Mol. Biol. Evol.* 11:9-21.

SWANEPOEL, R., BARNARD, C.D., MEREDITH, C.D., BISHOP, G., BRÜCKNER, G.K., FOGGIN, C.M. & HÜBSCHLE. O.J.B. 1993. Rabies in southern Africa. *Onderstepoort J. Vet. Res.* 60:32-346.

SWOFFORD, D.L. & OLSEN, G.J. 1990. Phylogeny reconstruction. In: *Molecular systematics*, (eds.) D.M. Hillis & C. Moritz. Sinauer Associates, Inc. Sunderland, Massachusetts.

SWOFFORD, D.L. 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.2d5. Illinois Natl. Hist. Surv., Champaign, IL.

TAKAHATA, N. & PALUMBI, S.R. 1985. Extranuclear differentiation and gene flow in the finite island model. *Genetics* 109:441-457.

TAYLOR, P.J. 1990. *Intraspesific systematics of the yellow mongoose Cynictis penicillata*. Ph.D thesis, University of Natal, South Africa.

TAYLOR, P.J., CAMPBELL, G.K., VAN DYK, D., WATSON, J.P., PALLETT, J. & ERASMUS, B.E. 1990. Genic variation in the yellow mongoose, *Cynictis penicillata* in Southern Africa. *S. A. J. Science* 86: 256-262.

TAYLOR, P.J. & MEESTER, J. 1993. Morphometric variation in the yellow mongoose, *Cynictis penicillata* (Cuvier, 1829) (Carnivora: Viverridae), in Southern Africa. *Durban Mus. Novit.* 18:37-71.

VAWTER, L. & BROWN, W.M. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* 234:194-196.

WAYNE, R.K., BENVENISTE, R.E., JANCZEWSKI, D.N. & O'BRIEN, S.J. 1989. Molecular and biochemical evolution of the Carnivora. In: *Carnivore Behaviour, Ecology and Evolution*, (ed.) J.L. Gittleman. Cornell University Press, Ithaca.

WAYNE, R.K. & JENKS, S.M. 1991. Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. *Nature* 351:565-568.

WAYNE, R.K., LEHMAN, N., ALLARD, M.W. & HONEYCUTT, R.L. 1992. Mitochondrial DNA variability of the grey wolf: Genetic consequences of population decline and habitat fragmentation. *Conserv. Biol.* 6:559-569.

WAYNE, R.K., MEYER, A., LEHMAN, N., VAN VALKENBURGH, B., KAT, P.W., FULLER, T.K., GIRMAN, D. & O'BRIEN, S.J. 1990. Large sequence divergence among mitochondrial DNA genotypes within populations of eastern African black-backed jackals. *Proc. Natl. Acad. Sci. USA.* 87:1772-1776.

WAYNE, R.K., VAN VALKENBURGH, B., KAT, P.W., FULLER, T.K., JOHNSON, W.E. & O'BRIEN, S.J. 1989. Genetic and morphological divergence among sympatric canids. *J. Heredity* 80:447-454.

WENHOLD, B.A. 1990. The ethology and social structure of the yellow mongoose *Cynictis penicillata*. MSc thesis, University of Pretoria, South Africa.

WILKINSON, G.S. & CHAPMAN, A.M. 1991. Length and sequence variation in evening bat D-loop mtDNA. *Genetics* 128:607-617.

WILSON, A.C., SARICH, V.M. & MAXSON, L.R. 1974. The importance of gene rearrangements in evolution: Evidence from studies of rates of chromosomal, protein, and anatomical evolution. *Proc. Natl. Acad. Sci. USA.* 71: 3028-3030.

WOZENCRAFT, W.C. 1989a. The Phylogeny of the recent Carnivora. In: *Carnivore Behaviour, Ecology and Evolution*, (ed.) J.L. Gittleman. Cornell University Press, Ithaca.

WOZENCRAFT, W.C. 1989b. Classification of the recent Carnivora. In: *Carnivore Behaviour, Ecology and Evolution* (ed.) J.L. Gittleman. Cornell University Press, Ithaca.

ZUMPT, I. 1976. The yellow mongoose *Cynictis penicillata* as a latent focus of rabies in South Africa. *J. S.A. Vet Ass.* 47: 211-213.

APPENDIX 1

GAZETTEER

LOCALITY	COORDINATES
Baberspan Nature Reserve	26°34'S 25°38'E
Bloemfontein	29°06'S 26°14'E
Bloemfontein Glen	28°57'S 26°20'E
Chelmsford Nature Reserve	27°45'S 29°57'E
Ermelo	26°31'S 30°00'E
Ghanzi	21°30'S 21°50'E
Kalahari Gemsbok National Park	25°30'S 20°50'E
Karoo National Park	32°30'S 22°30'E
Kimberley	28°44'S 24°46'E
Kroonstad	27°40'S 27°14'E
Kuruman	27°27'S 23°26'E
Pretoria	25°39'S 28°11'E
Queenstown	31°54'S 26°53'E
Riemvasmaak	28°27'S 20°19'E
Sandveld Nature Reserve	28°40'S 16°30'E
Stellenbosch	30°56'S 18°52'E
Tussen-die-Rivieren Game Farm	30°45'S 26°10'E
Van Zylsrus	26°26'S 22°12'E
Victoria West	31°30'S 23°11'E
Willem Pretorius Nature Reserve	28°30'S 27°10'E
Windhoek	22°34'S 17°06'E

APPENDIX 2

Accession numbers of specimens used in this study. The museum collections in which they occur are TM: Transvaal Museum, Pretoria; HW and MC: McGregor Museum, Kimberley; DM: Durban Natural Science Museum, Durban. Specimens 5131-5160 have been deposited in the National Museum, Bloemfontein.

Specimen number	Museum number	Locality
1	TM43762	Baberspan
2	TM43763	Baberspan
3	TM43764	Baberspan
4	TM43765	Baberspan
5	TM43766	Baberspan
6	Not available	Ermelo
7	Not available	Ermelo
8	Not available	Ermelo
9	Not available	Kuruman
10	Not available	Kuruman
11	Not available	Kuruman
12	Not available	Kroonstad
13	Not available	Kroonstad
14	Not available	Bloemfontein
15	Not available	Bloemfontein
16	Not available	Queenstown
17	Not available	Queenstown
18	TM39215	Pretoria
19	TM39265	Pretoria
20	TM39299	Pretoria
21	TM39406	Pretoria
22	TM39436	Pretoria

Specimen number	Museum number	Locality
23	TM39437	Pretoria
24	TM39448	Karoo NP
25	TM39937	Windhoek
26	TM39947	Windhoek
27	TM39948	Windhoek
28	TM39949	Windhoek
29	PT110	Victoria West
30	PT111	Victoria West
31	5131	Kroonstad
32	5132	Kroonstad
33	5158	Bloemfontein Glen
34	5159	Bloemfontein Glen
35	5160	Bloemfontein Glen
36	HW4784	Riemvasmaak
37	DM1620	Chelmsford NR
38	DM1621	Chelmsford NR
39	DM1622	Chelmsford NR
40	Not available	Sandveld NR
41	Not available	Sandveld NR
42	Not available	Tussen-die-Rivieren GF
43	Not available	Willem Pretorius NR
44	Not available	Ghanzi
45	MC539	Kimberley
46	MC540	Kimberley
47	TM44731	Van Zylsrus
48	Not available	Kalahari Gemsbok National Park
49	Not available	Kalahari Gemsbok National Park
50	Not available	Stellenbosch

APPENDIX 3

Nucleotide sequences of a 482 bp region of the mitochondrial DNA major non-coding region for five yellow mongoose specimens. The nucleotide sequences were also aligned to two outgroup species, *Canis lupus* and *C. aureus*. The 81 bp insert detected within the yellow mongoose corresponds to positions 284 to 364.

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                    10         20         30         40         50
B4          GCTAAAGCTCCACTATCAGCACCCAAAGCTGAAGTTCTTTTTTAAACTAT
B13         GCTAAAGCTCCACTATCAGCACCCAAAGCTGAAGTTCTTTTTTAAACTAT
B24         GCTAAAGCTCCACTATCAGCACCCAAAGCTGAAGTTCTTTTTTAAACTAT
B25         GCTAAAGCTCCACTATCAGCACCCAAAGCTGAAGTTCTTTTTTAAACTAT
B37         GCTAAAGCTCCACTATCAGCACCCAAAGCTGA?GTTCTTTTTTCAACTAT
DL-CLU24    GCTCTTGCTCCACCATCAGCACCCAAAGCTGAAATTC-TTCTTAAACTAT
DL-CAU      GCTCTTGCTCCACCATCAGCACCCAAAGCTGAAATTC-TTCTTAAACTAT

                    60         70         80         90        100
B4          TCCCTGCAATACCACAATACTACCCCAAACTTTCGCTATTCATATATTGC
B13         TCCCTGCAATACCACAATCCTACCCCAAACTTTCGCTATTCATATATTGC
B24         TCCCTGCAATACCACAATCCTACCCCAAACTTTCGCTATTCATATATTGC
B25         TCCCTGCAATACCACAATCCTACCCCAAACTTTCGCCATTCATATATTGC
B37         TCCCTGCAATACCACAATCCTACCCCAAACTTTCGCTATTCATATATTGC
DL-CLU24    TCCCTGGAA----------CCCC---CTTAC---ATTCATATATTGG
DL-CAU      TCCCTGACA----------CCCC---CTTAC---ATTCATATATTGG

                    110        120        130        140        150
B4          ATGACATAGTACTGTGCTTGCCCAGTATGTCCTATCTTACCCTAACTAGT
B13         ATGACATAGTACTGTGCTTGCCCAGTATGTCCTATCTTACCCTAACTAGT
B24         ATGACATAGTACTGTGCTTGCCCAGTATGTCCTATCTTACCCTAACTAGT
B25         ATGACATAGTACTGTGCTTGCCCAGTATGTCCTATCTTACCCTAACTAGT
B37         ATGACATAGTACTGTGCTTGTCCAGTATGTCCTATCTTACCCTAACTAGT
DL-CLU24    ACTAGCC-TTACTGTGCTATGTCAGTATCTCC-----------------
DL-CAU      ACCA-CCTCTACTATGCTATGTCAGTATCTCC-----------------

                    160        170        180        190        200
B4          ACACAACGTGTACATTACATGTTATTTAATATATGCTATGTATATCGTGC
B13         ACACAACGTGTACATTACATGTTATTTAATATATGCTATGTATATCGTGC
B24         ACACAACGTGTACATTACATGTTATTTAATATATGCTATGTATATCGTGC
B25         ACACAACGTGTACATTACATGTTATTTAATATATGCTATGTATATCGTGC
B37         ACACAACGTGTACATTACATGTTATTTAATATATGCTATGTATATCGTGC
DL-CLU24    --A-AA-----A-A----ATTTTCCT--CCCTATG-TACGTCGTGCATTA
DL-CAU      --A-AA-----A-A----ATCCTTCTTTCCCTCCCCTATGTACGTCGTGC
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