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Genetic variation among South African hares (*Lepus* spec.) as inferred from mitochondrial DNA and microsatellites

by

Ute Kryger

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Supervisor: Dr. P. Bloomer
Co-supervisor: Prof. T.J. Robinson

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SUMMARY

The inter- and intraspecific taxonomy of the genus *Lepus* is highly controversial. Since these animals play an important ecological role as prey species, their conservation is of high priority. A prerequisite for adequate management strategies is the precise knowledge of taxonomic borders and geographic distributions of the taxa in question. Especially in African hares the clear delimitations of the species and subspecies are still unresolved. In an attempt to obtain a clearer understanding of the evolutionary history, the phylogenetic relationships and the detection of conservation relevant units in African hares, a molecular investigation using mitochondrial DNA sequence data and information from six microsatellite loci was initiated.

In this study, the phylogenetic analyses of DNA sequence data of the mitochondrial control region and the cytochrome *b* gene identified 2 major maternal lineages within South African hares that were further subdivided into two clusters each. The nuclear genetic structure revealed by six dedicated microsatellite loci confirmed this subdivision. While the mitochondrial diversity was characterized by high sequence divergences and haplotype diversities, the nuclear variation seemed more moderate with relatively low F_{ST} and R_{ST} values. Using the information content of the six microsatellite loci developed for this project, the results of assignment tests strongly supported the validity of the four genetic lineages. The levels of of cytochrome *b* sequence divergences among the four clusters suggested that they may warrant distinct species status (applying the phylogenetic species concept). The data are suggestive of two species groups consisting of two sister species each: A “saxatilis” group with *Lepus saxatilis sensu stricto* and another species (possibly *L. victoriae*), and a “capensis” group with *L. capensis s. str.* and another, yet to be described species. Applying a molecular clock for cytochrome *b*, the historical dates for the separation of the South African hares from European outgroup species and the split between the two South African species groups were calculated as 4.84 and 3.45 million years ago, respectively. The divergence dates between the sister species was 1.09–1.45 million years ago.

Following the biological species concept, the two major clades represent two species: *L. saxatilis sensu lato* and *L. capensis s. l.*, that are both subdivided into two geographically separated phylogroups, one in the southern parts of the country and one in the northern. These intraspecific lineages meet the criteria of evolutionarily significant units and should be considered separately in conservation actions. The evolutionary history of scrub and Cape hares in South Africa has been influenced mainly by contiguous range expansions and sudden population size expansions during the Pleistocene climate changes.

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"And let's not forget: situations causing headache to taxonomists may be interesting moments in the evolution of species." (Angermann 1983)

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LIST OF ABBREVIATIONS

AMOVA	Analysis of molecular variance
bp	Basepairs
BP	Before present
CI	Consistency index
CR	Control region
CR-I	5' terminal of the control region
CRE	Contiguous range expansion
D_c	Clade distance
d.f.	Degrees of freedom
DI	Decay index
D_n	Nested clade distance
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
ESU	Evolutionarily significant unit
FRA	Fragmentation
H	Haplotype diversity
H_E	Expected heterozygosity
HKY85	Hasegawa-Kishino-Yano (1985) model of nucleotide substitution
H_O	Observed heterozygosity
HT	Haplotype
HWE	Hardy-Weinberg equilibrium
IAM	Infinite allele model
IBD	Isolation by distance
IUCN	International union for the conservation of nature
λ	Rate of nucleotide substitution per site per lineage per year
LDC	Long distance colonization
μ	Mutation rate per generation per nucleotide site
Ma	Million years ago

ML	Maximum Likelihood
mM	Millimolar
μM	Micromolar
MP	Maximum parsimony
mtDNA	Mitochondrial DNA
MU	Management unit
Myr	Million years
ν	Mutation rate per generation per haplotype
NCA	Nested clade analysis
N_e	Effective population size
ng	nanogram
NJ	Neighbor-joining
OUT	Operational taxonomic unit
π	Nucleotide diversity
PAUP	Phylogenetic analysis using parsimony
PCA	Principal component analysis
PCR	Polymerase chain reaction
pmol	picomol
RGF	Restricted gene flow
RI	Retention index
rRNA	Ribosomal ribonucleic acid
s	Polymorphic sites
s. l.	*sensu lato* (in the broader sense)
SMM	Stepwise mutation model
s. str.	*sensu stricto* (in the narrow sense)
τ	Moment estimator to expansion time
θ	Mutation parameter
Ti	Transition
TMRCA	Time to the most recent common ancestor
Tv	Transversion

Chapter 1

General Introduction

1.1 Background information on the study animals

The order Lagomorpha

The order Lagomorpha consists of two families. The family Ochotonidae comprises the pikas from North America and Eurasia. The cosmopolitan family Leporidae consists of the rabbits, jackrabbits and hares with 11 living and 21 fossil genera. Representatives of the two families show different and sometimes diagnostic physical features. The monotypic Ochotonidae (with a single extant genus, *Ochotona*) are small animals (some species weigh less than 100 g) with equally long fore- and hindlimbs, round ears, short nasals and skulls without supraorbital bones. They have 26 teeth whereas the Leporidae have 28 teeth. The latter are larger, with some hares exceeding five kilograms (Chapman & Flux 1990). The hindlimbs of the leporids are longer than their forelimbs, which is interpreted as an adaptation for rapid cursorial locomotion, flight speed may reach 80 km per hour in some hares. The ears are elongated, the eyes are large (both interpreted as adaptations to their generally nocturnal habits), and the skull is characterized by a long nasal region and prominent supraorbital bones (Angermann *et al.* 1990).

All true hares (jackrabbits and hares) belong to the genus *Lepus* (Chapman and Flux 1990). Rabbits fall within the genera *Oryctolagus* (European rabbit), *Brachylagus* (pygmy rabbit), *Nesolagus* (Sumatran rabbit), *Sylvilagus* (cottontails), *Caprolagus* (Hispid hare, actually a real rabbit), *Pentalagus* (Amami Rabbit), *Romerolagus* (volcano rabbit), *Poelagus* (Bunyoro rabbit), *Bunolagus* (riverine rabbit), and *Pronolagus* (red rock rabbit).

Lagomorphs have not adapted to any particular environment; representatives of the order have been extraordinarily successful in occupying the most diverse of habitats

throughout the world (Colbert 1980). Lagomorphs in general practice re-ingestion, that is they eat soft faecal pellets that they have produced during their resting periods. Through this process they recycle important minerals like calcium and phosphorus, get access to the Vitamin B and K manufactured in their hindgut, and maximize the energy gain from the food (Duthie 1997). Schneider (1990) interprets this metabolic peculiarity as a major factor enabling lagomorphs to settle in the most widely varied habitats.

Taxonomic position of lagomorphs

Lagomorphs were originally classified as a suborder of rodents (Duplicidentata) because they possess a pair of enlarged ever-growing re-curved incisor teeth well adapted for gnawing. In 1912, Gidley recognized the order Lagomorpha as being distinct from the order Rodentia based on the presence of a second set of incisors (so-called "peg" teeth) directly behind the upper front incisors. Consequently, lagomorphs were placed together with rodents as the two major groups of gliriform mammals sharing a common ancestor (Gregory WK 1910; Simpson GG 1945; Chuan-Kuei *et al.* 1987). Novacek (1987) regarded the gliriform incisors (one of the proposed synapomorphies defining the cohort Glires) of lagomorphs and rodents as convergent. But Pesole *et al.* (1991) supported the monophyly of the superorder Glires based on mitochondrial data and Novacek (1992) accepted Glires as a supraordinal clade on the basis of combined morphological and molecular data sets.

In a series of four-taxon analyses using 91 protein sequences, Graur *et al.* (1996) came to the conclusion that the closest relatives of lagomorphs are the primates. Halanych (1998) criticized the lack of evidence from morphology, embryology or fossil records to support the sister relationship of primates and lagomorphs and instead emphasized the fossil evidence for a lagomorph-rodent association. Furthermore, this author pointed out that the study of Graur *et al.* (1996) introduced biases through missing and uninformative data and long branches, and that these biases were amplified by underrepresentation of taxa. By excluding uninformative and partially missing data from the analyses and by excluding the outgroup and adding more taxa, Halanych (1998) found that the protein sequence data used by Graur *et al.* (1996) actually support the Glires concept with high

bootstrap values for a lagomorph-sciurognath cluster. In contrast, De Jong (1998) quoted several authors rejecting the grouping of lagomorphs and rodents on the basis of mitochondrial protein data. Springer *et al.* (1999) analyzed four mitochondrial and four nuclear genes for 11 eutherian orders and found no significant support for the Glires clade, but they only included the European rabbit as a representative for the lagomorphs and the mouse as the sole representative for rodents. Waddell *et al.* (1999) retrieved support for the traditional Glires clade by adding sequences from the dormouse to the analyses and found that its closest relatives are the ferungulates, not the primates. Recent molecular phylogenetic analyses based on 19 nuclear and three mitochondrial gene sequences confirmed the validity of the sister-relationship of Lagomorpha and Rodentia (Murphy *et al.*2001 a, b).

Evolution of lagomorphs

Different hypotheses exist regarding the evolutionary origin of lagomorphs. Some authors affiliate them with the Triconodonta of the early Mesozoic; others favor the Protoinsectivora, Analgida (extinct relatives of the tree shrews) or original ungulates from the late Mesozoic as probable ancestors (Schneider 1990). According to Carroll (1988), lagomorphs have been separate from their nearest relatives as an independent order for at least 60 million years. Colbert (1980) interpreted Mongolian fossils from the upper Paleocene as belonging to the genus *Eurymylus* and took this to be the earliest fossil evidence of rabbits. Angermann (1973) and Schneider (1990) supported this view and considered either *Mimolagus* or *Hsiuannania* (both Paleocenic and found in Asia) as the earliest true representatives of hares or rabbits.

Chapman & Flux (1990) dated the separation of the ochotonids from the leporids as early as the Eocene, 50 million years ago (Ma); Halanych & Robinson (1999) placed this separation some 30-40 Ma. The later authors also highlighted the absence of synapomorphies in paleontological and cytological data and inferred that the majority of leporid genera (including *Lepus*) must have originated from a single, brief and rapid radiation event. These authors constructed a molecular phylogeny of the leporids analyzing mitochondrial cytochrome *b* and 12S ribosomal DNA sequences. The internal

branches in this molecular phylogeny are very short, which the authors interpreted as additional evidence for a very rapid diversification event. Chapman & Flux (1990) postulated that a rapid radiation occurred in the middle to late Pleistocene, which is in agreement with Angermann (1973) who suggested that lagomorphs, in the narrow sense, have speciated and spread throughout the world in the Pliocene and Pleistocene. In contrast, the molecular data from Halanych & Robinson (1999) proposed a much earlier date for this radiation at approximately 12-16 Ma (Miocene).

The first ochotonids appeared in the mid-Oligocene of Asia and apparently also spread to Europe during this epoche (Schneider 1990). Following the opening of terrestrial connections, the ochotonids entered Africa in the Miocene (Carroll 1988). Lavocat (1978) reported ochotonid fossils from that period, Astrolagomys (in the Namib) and Kenyalagomys (in East Africa). As reported by Carroll (1988), primitive hares are well documented from the Eocene in Eastern Asia (*Lushilagus*, *Shamolagus*) and North America (*Mytonolagus*); Angermann (1973) classified these species as Protolagomorphs (Palaeolaginae). Leporids supposedly arrived in Africa in the Pliocene (Carroll 1988). According to Walker (1964), the oldest fossils of true representatives of the genus *Lepus* date back to the early Pleistocene which is concordant with Lavocat (1978) who descibed Pleistocene fossils of *Pronolagus* and *Lepus* in Africa. Analyzing mitochondrial DNA (mtDNA) restriction sites, Biju-Duval *et al.* (1991) suggested that the most recent common ancestor of the genera *Lepus*, *Oryctolagus*, and *Sylvilagus* lived 6-8 Ma.

Natural history of hares

Hares are small to medium-sized herbivores that constitute the base of many predator-prey systems (Chapman & Flux 1990). They are generally nocturnal, during the day they lie up in forms. In cool weather or when chased up from their forms they can also be seen during the daytime (Duthie 1997). Hares enjoy a complete field of vision (360 degrees) due to the lateral position of their eyes. However, their visual acuity is rather poor and in adaptation to their nocturnal activity patterns, they rely more on their hearing and sense of smell than on sight (Schneider 1990). Hares use secretions from glands, urine and faeces as transmitters of olfactory messages. They communicate vocally by producing

high-pitched contact calls as well as ultrasonic noises made by rubbing their lower incisors against their peg teeth (Schneider 1990).

Hares generally have high reproduction rates and high rates of population turnover, with mortality rates sometimes reaching up to 90% annually. Mortality is mostly the consequence of diseases, parasites, road traffic, and predation. The average annual reproduction per female hare is about ten young for most species (Flux & Angermann 1990; Penzhorn *et al.* 1993). Both sexes do apparently not breed in their year of birth. Their reproductive behaviour is characterized by several males chasing a single female who may rebuff them by boxing.

Despite the morphological similarities, there are several important differences between hares and rabbits. Robinson (1981) found a difference in the number of chromosomes with *Lepus* consistently possessing a karyotype of 2n = 48, whereas the karyotype in rabbits is more variable (e.g. 2n = 42 in *Pronolagus*, and 2n = 52 in *Sylvilagus transitionalis*); the karyotype 2n = 48 is hypothesized to be the ancestral state. The young of the hares, so-called leverets, are born fully furred with open eyes and ears and are mobile within 48 hours after birth. Hares do not build nests or burrows. In contrast young rabbits, kittens, are born naked and with plugged ears and closed eyes. Female rabbits (does) construct nests and care for their young for up to three weeks. Furthermore, hares are cursorial and possess dark red flesh with long-fibred muscles, whereas rabbits are more specialized for digging, generally run only short distances and have light, short-fibred flesh (Schneider 1990). The ears of hares always have a patch of black at their tip (Schneider 1990) and their length is significantly larger than in rabbits. Galis (1996) interpreted the long and heavy ears of hares as an adaptation to saltatory movement: when the hare hits the ground at high speed the anterior part of the head is relatively displaced resulting in shock absorption; through flipping the ears upright a pulling force is created which restores the original position of the anterior part of the head.

In hares the population sizes are regulated by extreme fluctuations in the environment, depletion of food resources, and dispersal which may be innate or brought about by density dependent limitation of resources (Chapman & Flux 1990). Single hares can move distances of up to 20 km per night, and Schneider (1990) reports on individuals moving distances of up to 400 km. Whole populations can shift hundreds of kilometers in

response to environmental changes or in search of better feeding grounds and there are numerous reports of "hare migrations" where large populations travel together (Angermann *et al.* 1990; Schneider 1990). Regular population density cycles have been documented for a number of hares and jackrabbits, but have not been reported in African species (Schneider 1990).

The preferred habitat of *Lepus* is open country with interspersed rocks, bushes or shrubs that provide cover and protection from predators (Flux & Angermann 1990). Hares are solitary animals. Individual home ranges often overlap on popular feeding grounds.

Number and identity of hare species in South Africa

The number of recognized species within the genus differs from approximately 12 to over 30. The validity of many described species is a topic of debate and the evolutionary pathways within the genus are still confused and ill-defined (Flux & Angermann 1990). This is reflected in the South African context. Petter (1972) reported on three species, namely *L. capensis*, *L. saxatilis*, and *L. crawshayi*. Angermann (1973) recognized *L. capensis*, *L. saxatilis*, and *L. whytei*, whereas Kingdon (1974) only accounted for *L. capensis* and *L. crawshayi*. Robinson (1981), Robinson & Dippenaar (1983, 1987), Skinner & Smithers (1990), and Duthie (1997) agreed with Roberts (1951) and only recognized *L. capensis* and *L. saxatilis*. Flux and Angermann (1990) identified *L. capensis*, *L. saxatilis* and *L. victoriae* as occurring in South Africa, but doubted the validity of many of the described subspecies.

Subspecies of the scrub hare and the Cape hare

Roberts (1951) emphasized the considerable geographical variation in color shades and size for both South African hare species reported in his work (*L. saxatilis* and *L. capensis*). Based on these morphological traits, he described 14 subspecies for *L. capensis* (Table 1.1) and 17 for *L. saxatilis* (Table 1.2), but cautioned that there

actually appear to be distinct species hidden in the subspecies complexes of both groups. Contrasting to the exclusively westerly distribution of the Cape hare given by most other authors, Roberts (1951) and Petter (1972) referred to a subspecies of the Cape hare (*L. capensis ermeloensis* Roberts, 1932) as occuring as far east as Ermelo, and over larger parts of the former Eastern Transvaal (now Mpumalanga).

Of Roberts' (1951) subspecies for the scrub hare, Petter (1972) confirmed only six (*L. s. saxatilis*, *L. s. megalotis*, *L. s. aurantii*, *L. s. albaniensis*, *L. s. chiversi*, *L. s. orangensis*), thus restricting the distributional limits of *L. saxatilis* to the southwestern half of South Africa, a view shared by Angermann (1973) and Flux & Angermann (1990). Petter (1972) interpreted the other 11 forms as subspecies of *L. crawshayi*, Crawshay's hare, on the basis of the characters ears shorter than 130 cm and cranium shorter than 92 mm.

Table 1.1 Subspecies of the Cape Hare occurring in southern Africa following Roberts (1951).

Subspecies	Distribution
L. c. bedfordi Roberts, 1932	Kalkbank, Pietersburg district, former northern Transvaal
L. c. capensis Linn., 1758	Western Cape Province & eastwards to Oudtshoorn
L. c. carpi Lundholm, 1955	Oropembe, Kaokoveld, Namibia
L. c. centralis Thomas, 1903	Central Karoo
L. c. ermeloensis Roberts, 1932	Ermelo, Carolina & Wakkerstroom district
L. c. granti Thomas & Schwann, 1904	Northwestern Cape Province to Port Nolloth
L. c. hartensis Roberts, 1932	Vaal River northwards to Molopo River
L. c. kalaharicus Dollman, 1910	Kalahari desert and Great Namaqualand
L. c. langi Roberts, 1932	Nkate River, northern Botswana
L. c. mandatus Thomas, 1926	Northwest of Keetmanshoop, Namibia
L. c. narranus Thomas, 1926	Near Walvis Bay, Namibia
L. c. ochropoides Roberts, 1929	Burghersdorp, Eastern Cape Province
L. c. ochropus Wagner, 1844	Former Southern Transvaal & northern Orange Free State
L. c. vernayi Roberts, 1932	Kroonstad to Hopetown, Former Orange Free State

Table 1.2 Subspecies of the scrub hare based on body measurements and coloration as suggested by Roberts (1951).

Subspecies	Distribution and special features
L. s. saxatilis F. Cuvier, 1823 common name: Ribbokhaas	Southern and western districts of the former Cape Province; the largest of the South African hares
L. s. megalotis Thomas and Schwann, 1905	Upper Karroo, from Murraysburg to little Namaqualand; very large animal with very long ears
L. s. aurantii Thomas and Hinton, 1923	Orange River near Upington through Great Namaqualand to Rehoboth in Namibia
L. s. albaniensis Roberts, 1932	Eastern Cape Province, from the Albany district eastwards to King Williamstown and northwards to Middelburg
L. s. chiversi Roberts, 1929	Known only from the type locality Rouxville, but supposedly extending in to the karooveld areas of the south-eastern corner of the Former Orange Free State.
L. s. orangensis Kolbe, 1948	Recorded only in the Hoopstad district, but supposedly occurring over the former northwestern Orange Free State.
L. s. subrufus Roberts, 1913	Former Transvaal highveld, from the escarpment westwards to the Potchefstroom district and probably Rustenburg and Wolmaransstad
L. s. zuluensis Thomas and Schwann, 1905	Former Zululand to former Eastern and Northern Transvaal; a dark animal with short ears and a small skull
L. s. bechuanae Roberts, 1932	Southern Bechuanaland and former western Transvaal
L. s. chobiensis Roberts, 1932	Northern Bechuanaland, bordering the Chobe River
L. s. ngamiensis Roberts, 1932	Ngamiland, from Lake Ngami to Mababe Flats
L. s. damarensis Roberts, 1926	Damaraland
L. s. khanensis Roberts, 1946	Type locality Khan River near Karibib, supposedly extending into the Namib along the Khan and Swakop Rivers
L. s. herero Thomas, 1926	Ovamboland and Kaokoveld, Omaruru district, Grootfontein district, and southwards through the dry Kalahari Desert
L. s. micklemi Chubb, 1908	Highland area of Zimbabwe
L. s. nigrescens Roberts, 1932	Coastal lowlands of Mozambique, near Beira, a very dark and small form
L. s. gungunyanae Roberts, 1914	Type locality in the Matibi district of Zimbabwe

1.2 Biogeography of African small mammals

Biogeography is the study of organismal diversity in time and space. The interpretation of the speciation and distribution of organisms in terms of isolation and dispersal events in response to climatic and vegetational changes is the main focus of historical biogeography (Grant 1993).

Relevant for this study are those changes that occurred during the Pleistocene (1.8-0.11 Ma) and the Holocene (0.11 Ma to the present). Shifts in climate and the resulting impact on vegetation may be deducted from oxygen-isotope records obtained from deep-sea sediment cores and cave deposits (Tyson & Preston-Whyte 2000). During the glacial maxima, huge sheets of ice covered large continental areas in the northern hemisphere. Even though Africa was relatively ice-free during the Pleistocene, global glaciation led to shifting rainfall patterns and the pronounced aridification in eastern and southern Africa (Grant & Leslie 1993). Thus the Quaternary climatic changes on the African continent consisted of cycles of cold/arid and warm/moist phases which, in turn, led to cycles of contraction and expansion of major vegetational zones as deducted by palynological (pollen) records (Grubb 1978). The African fossil record suggests that the paleoenvironmental shifts towards more arid, open conditions near 2.8 Ma, 1.7 Ma, and 1.0 Ma were coincident with major evolutionary steps in African hominids and other vertebrates. The vegetation shifting from closed forest canopies to open savanna, as well as reduced and more seasonally contrasted precipitation, was accompanied by the replacement of humid-adapted taxa by those that were arid-adapted (De Menocal 1995). This is thought to have had profound effects on the evolution of the African mammalian fauna and their distribution patterns (Coe & Skinner 1993). Recurrent habitat fragmentation and isolation events may have led to sub-speciation, speciation or extinction (Ewer & Cooke 1964; Grant & Leslie 1993). Renewed dispersal during times of habitat expansion can lead to secondary contact between formerly allopatric species or subspecies, and can involve varying degrees of genetic introgression (intrinsic hybridization and differential gene flow between two closely related taxa) in hybrid zones (Barton *et al.* 1983; Hewitt 1988, 2001; Barton & Hewitt 1989). The discovery of "incompatible" characters, such as particular mitochondrial haplotypes, against an

otherwise mostly "normal" genetic or morphological background can be seen as evidence for introgression (Brower *et al.* 1996). In 1978, Grubb noted that until then no such hybrid zones had been described for African small mammals, a group characterized by rapid speciation and the presence of sympatric sibling species. It is believed that many savanna species are of forest origin, and speciation in these cases involved an ecological transition from mesic, predictable habitats toward arid, unpredictable habitats (Grubb 1978).

In more recent times, the southwards migration of pastoralists and their domestic stock, over the last 1500 years, further affected the distribution of major vegetation types and thus the range of animal species in southern Africa (Coe & Skinner 1993). This effect might have been especially pronounced for so called "culture followers" such as hares (Angermann 1983).

1.3 Phylogeography

Phylogeography is a relatively new biological discipline concerned with the processes and principles determining the geographic distributions of genealogical lineages (Avise *et al.* 1987; Avise 1998, 2000). Edwards & Beerli (2000) defined phylogeography as the application of molecular methods to the biogeography of a single species. Avise *et al.* (1987) emphasized the integrative nature of the discipline as a contact zone between population genetics and phylogenetics. Avise (1998, 2000) identified the heuristic place of "phylogeography" as the "sister-discipline" to ecogeography, both being sub-disciplines of biogeography. Ecogeography, on one hand, emphasizes the influence of contemporary ecological pressures (especially natural selection) on the geographic distribution of organismal traits. Where selection-mediated responses to environmental gradients are consistent over many different species, ecogeographic rules (e.g. Bergmann's rule, Allen's rule etc.) are constituted to describe these phenomena. Phylogeography, on the other hand, emphasizes the influence of historical factors such as dispersal events and periods of vicariance on the geographic distribution of gene lineages. Historical partitioning can be discovered by phylogeographic investigations if the

analyzed gene lineages were strictly fitness-neutral (Avise 2000). The great majority of phylogeographic research so far has dealt with historical, phylogenetic components of the spatial distribution of mitochondrial gene lineages within and among closely related species (Avise 2000).

Phylogeographic hypotheses

Avise *et al.* (1987) and Walker & Avise (1998) formulated three hypotheses as the conceptual framework for phylogeographic studies. They postulated that most species display a significant phylogeographic structure with members of different geographic populations occupying recognizable genealogical branches of the intraspecific pedigree. Furthermore, they attributed a limited ("shallow") phylogeographic structure in a species to a life history characterized by high dispersal capabilities and attendant imperviousness to historical (biogeographic) barriers to gene flow. And lastly, they hypothesized that major monophyletic units within a species distinguished by large genealogical gaps usually arise from long-term extrinsic (biogeographic) barriers to gene flow. As suggested by Avise *et al.* (1987) and Avise (2000), the genetic architectures of extant species are the results of historical biogeographic factors as well as contemporary ecologies and behaviors of the organisms under investigation. Such genetic architectures can be classified into five major phylogeographic categories characterized by the depth of the gene tree and the geographic distribution of the lineages (see Avise 1987). But Avise (2000) cautioned that it might be difficult to distinguish precisely between Categories III-V.

Methods in phylogeography

The great majority of phylogeographic work has been done utilizing mtDNA as the genetic marker of choice, but Avise (1998) emphasized the importance of including nuclear gene genealogies in future phylogeographic studies. Mitochondrial DNA only provides insights into the matrilineal pathways of ancestry, which represent only a small

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fraction of the complete historical record within the pedigree of a sexually reproducing organism. Consequently, the patrilineal pathways should also be investigated using either Y-chromosome located markers (in mammals) or autosomal markers such as microsatellites whose alleles are transmitted via both genders.

Mitochondrial DNA. The mitochondrial DNA in higher animals is a double-stranded, covalently closed circular molecule of approximately 16-20 kilobases in length (Avise 1986). The mtDNA molecule hosts 37 genes: 13 messenger RNAs, two ribosomal RNAs, 22 transfer RNAs, and a noncoding region, the so-called control region (CR; Boore 1999). The rate of evolution of mtDNA seems to exceed that of single-copy nuclear DNA by a factor of 5-10 (Brown *et al.* 1979; Harrison 1989) with most changes being simple base substitutions. The higher mutation rate of mtDNA is explained by the lack of efficient repair mechanisms (Barton & Jones 1983). Most differences between closely related mitochondrial sequences consist of transitions. In intraspecific comparisons Ti/Tv ratios can be as high as 10-20. Over longer evolutionary time spans transversions also accumulate and the Ti/Tv ratios eventually drop below one. This is interpreted as an indication for mutational saturation (Simon *et al.* 1994) where the number of transitions is underestimated due to the prevalence of multiple Ti hits at the same sites.

Avise *et al.* (1979, 1987), Moritz *et al.* (1987), and Harrison (1989) identified mtDNA as an ideal molecular system for phylogenetic analysis because of a unique combination of attributes. Mitochondrial DNA is distinct and yet ubiquitously distributed. It has a comparatively simple genetic structure (no transposable elements, pseudogenes or introns). It is usually strictly maternally inherited excluding the possibility of recombination. However, Gyllensten *et al.* (1991) and Rawson & Hilbish (1995) documented paternal leakage in mice and blue mussels, respectively; while Eyre-Walker *et al.* (1999) and Dowton & Campbell (2001) suggested intramitochondrial recombination in humans. Furthermore, individuals are usually effectively haploid regarding the number of mtDNA types they carry. Due to the smaller effective population size in comparison with autosomal loci, the fixation of mitochondrial haplotypes occurs four times more rapidly than among nuclear alleles; thus low levels of gene flow that might still maintain nuclear panmixia may already lead to differentiation of

mitochondrial lineages in different local demes (Barton & Jones 1983). Brower *et al.* (1996) therefore pointed out that mtDNA seems to be the superior marker to apply to studies concerned with the relationship among closely related taxa. Harrison (1989) highlighted the considerable amount of mtDNA variation among individuals within and between populations, and emphasized the effectiveness of using mtDNA to address questions of population structure, patterns of intraspecific geographic variation, and recent evolutionary history such as colonization events and population bottlenecks.

Control Region. The rates of sequence evolution vary among the different mitochondrial genes and regions. Harrison (1989), Simon *et al.* (1994), and Baker & Marshall (1997) recognized the non-coding control region (CR) as the fastest evolving part of the mitochondrial genome therefore highlighting its suitability for investigating recently diverged taxa and relationships below the species level.

The mammalian mtDNA CR is responsible for regulating the replication and transcription of the mtDNA molecule (Boore 1999) and consists of three domains: the rapidly evolving A-rich left and right domains, and the central conserved domain (Taberlet 1996). The left domain (also 5' end or CR-I) contains sequence-stretches involved in the pausing of the synthesis of the nascent heavy strand. The C-G rich central conserved region regulates the replication of the heavy strand. The right domain (also 3' end or CR-II) includes the site of initiation of the heavy strand replication (Taberlet 1996). The two peripheral domains accumulate insertions and deletions and generate species-specific heterogeneity in both base composition and length (Saccone *et al.* 1991). Mignotte *et al.* (1990) found arrays of long (153 basepairs [bp]) and short (20 bp) repetitive sequences in the right domain of the European rabbit. The existence of such repetitive sequences has been confirmed for other lagomorph genera: namely for *Lepus* and *Sylvilagus* (Biju-Duval *et al.* 1991) and for *Ochotona* (Casane *et al.* 1997). Biju-Duval *et al.* (1991) described inter- and intraindividual length-polymorphism (heteroplasmy) due to variable numbers of these repeats.

The overall base composition of the control region in vertebrates is characterized by a paucity of G, with the light strand containing about 14% of G and about 28% of each of the other bases (Baker & Marshall 1997). Typical control region sequence divergences

among bird species range from 12-25%, while typical within-species sequence divergences range from 5-8% with high haplotype diversities. In all studies, transitions greatly outnumber transversions and alignment gaps, indicating that for the control region neither homology nor site saturation pose a problem at the intraspecific level (Baker & Marshall 1997). Pierpaoli *et al.* (1999) found interspecific divergences from 10-19% among European hares but only 2% between two Ethiopean hare species.

Numerous publications on vertebrates have proven the usefulness of this particular region for population studies: Among others these include Brown *et al.* (1993), Arctander *et al.* (1996), Berube *et al.* (1998), Eizirik *et al.* (1998), Fry & Zink (1998), Simonsen *et al.* (1998), Matsuhashi *et al.* (1999), Nagata *et al.* (1999), Nyakaana & Arctander (1999), and Birungi & Arctander (2000) applied CR sequences to infer population genetic structures of the White Sturgeon, Grant's gazelle, fin whales, neotropical cats, song sparrows, brown bears, African buffalo, sika deer, African elephants, and African kob antelope, respectively. Stepien & Faber (1998), Houlden *et al.* (1999), Davison *et al.* (2001), and Grau Nersting & Arctander (2001) used CR sequences for phylogeographic inferences in the walleye, the koala, the pine marten, and impala and greater kudu antelopes, respectively. Pierpaoli *et al.* (1999) successfully applied CR sequences to distinguish between different species of European hares. Douzery & Randi (1997) inferred phylogenetic relationships among members of the family Cervidae based on the entire control region (with exception of the repeat arrays and indels).

Cytochrome b. The cytochrome *b* gene is a mitochondrial protein coding gene containing phylogenetic information that has proved useful for assessing intraspecific up to intergeneric level associations (Kocher *et al.* 1989). In a recent study on pikas, Yu *et al.* (2000) indicated values ranging from 7-17% between well-accepted species, approximately 3-4% between sibling species, and below 2.9% within species. For leporids, interspecies divergences range from 3-12% among European hares (Pierpaoli *et al.* 1999) and from 2-12% among North American hares and jackrabbits (Halanych *et al.* 1999); intraspecific divergences are usually below 1% (Halanych *et al.* 1999). Santucci *et al.* (1998), Barber (1999a, b), Shields *et al.* (2000), and Ohdachi *et al.* (2001) inferred various vertebrate phylogeographies from cytochrome *b* sequences. Branco *et al.* (2000)

used the cytochrome *b* gene to investigate the phylogeography of the European rabbit. Halanych and Robinson (1999) found that cytochrome *b* is saturated on the inter-generic level in leporids. Yu *et al.* (2000) used cytochrome *b* sequences in order to reconstruct the phylogeny of the genus *Ochotona*. Fleischer *et al.* (2001) and Seddon *et al.* (2001) applied CR and cytochrome *b* sequences to infer the phylogeography of the Asian elephant and European hedgehogs, respectively.

Microsatellites. The term microsatellites describes tandem repeats of short (up to six nucleotides long) sequence motifs (Hancock 1999). These loci are powerful genetic markers because they are generally highly polymorphic. In comparison with allozymes and RAPDs (randomly amplified polymorphic DNA), microsatellites possess on average many more alleles per locus, furthermore they are codominant, neutral, and inherited in a Mendelian fashion (Jarne & Lagoda 1996). In eukaryotes, microsatellites are almost evenly distributed over the genome with exception of the non-coding regions and telomeres (Hancock 1999). The most common repeats seem to be poly (A)/poly (T) motifs, CA/TG repeats seem the most common dinucleotide repeats. Historically, only these CA/TG dinucleotide motifs have been called microsatellites whereas other repeats have been named simple sequences (Tautz 1989; Schlötterer *et al.* 1991) or STRs (short tandem repeats). Microsatellite loci may contain interruptions; the array is then called incomplete or imperfect. Loci that are made up of adjacent or contiguous tandem arrays of different motifs are called compound microsatellites (Hancock 1999).

Different alleles at a particular microsatellite locus are characterized by length differences due to variation in numbers of copies of the repeat. Following PCR amplification with locus-specific primers, the alleles are identified by their electrophoretic migration in comparison to a size standard. Haberl & Tautz (1999) drew attention to the possibility of mistakes in comparative allele sizing; they showed that amplifying the same loci for identical sample sets with matching but differently labeled primers sometimes leads to inaccurate allele size differences between the two systems. These authors recommended distinguishing “called” allele sizes from actual allele sizes. The "birth" of a microsatellite could be mediated by point mutations within cryptically simple sequences; equally the accumulation of point mutations within a microsatellite

itself is thought to lead to the "death" of this microsatellite (Hancock 1999; Ellegren 2000). Of practical concern are point mutations in primer annealing sites as they may cause the PCR amplification of that particular locus to fail. Such null alleles are reported to occur with a frequency of up to 15%, and Jarne & Lagoda (1996) estimated they occur in up to 25% of all loci.

Most studies suggest single-strand DNA slippage during replication as the predominant mutational process leading to different alleles by increasing or decreasing the current number of repeats (Levinson & Gutman 1987; Jarne & Lagoda 1996). However, evidence of other mutation mechanisms, such as unequal crossing-over, is accumulating (Hancock 1999; Ellegren 2000). There are three main models of microsatellite mutation that are currently the subject of debate (Jarne & Lagoda 1996). The infinite allele model (IAM; Kimura & Crow 1964) claims that each mutation creates a new allele at a rate u. The K-allele model (KAM; Crow & Kimura 1970) suggests that since K allelic states are possible, any mutation at a given allele occurs with the probability of $u/(k\text{-}1)$ and leads to any of the remaining k-1 allelic states. The stepwise mutation model (SMM; Ohta & Kimura 1973) proposes that any mutation adds or subtracts a single repeat unit to/from the original allele with a probability of u. In contrast to the first two models where all alleles differ equally from each other, the stepwise process of mutation is said to "have a memory", e.g. similar sized alleles are interpreted as less different in terms of mutational steps.

Zhu *et al.* (2000) mentioned that mutation rate and polymorphism increase with repeat number. The rates of mutation of microsatellites *in vivo* are estimated to range around 5×10^{-6} events per locus per generation in *Drosophila melanogaster* (Vazquez *et al.* 2000) to 10^{-3} in mice (Hancock 1999) and 2×10^{-3} per meiosis in human beings (Ellegren 2000). These high mutation rates together with high levels of polymorphism in natural populations have made microsatellites a very important class of genetic markers in population genetics (Jarne & Lagoda 1996; Stefanini & Feldman 2000). Among the many recent studies using microsatellites to address questions of population genetics are: Girman *et al.* (1993 & 2001), Estoup *et al.* (1995a, 1998, 1999, 2000), Gaggiotti *et al.* (1999), Garza & Williamson (2000), Goossens *et al.* (2000), Leblois *et al.* (2000), Pope *et al.* (2000), Spencer *et al.* (2000), Gerlach & Hoeck (2001), and Goodman *et al.* (2001).

1.4 Objective of this study

The main purpose of this study was to elucidate the phylogeography and evolutionary history of the scrub hare, and to a lesser extent, the Cape hare. In addition, this study strived to provide molecular genetic evidence for the resolution of the inter- and intraspecific taxonomy of South African hares in general.

The following null hypotheses were addressed:

H1 – There are two hare species present in South Africa, *L. saxatilis* and *L. capensis*, that each comprises of 10 subspecies.
H2 – Both South African hare species show a shallow phylogeographic pattern due to their high vagility.
H3 – South African hares are relatively young taxa with their origin in the Pleistocene.
H4 – Following the expansion of their habitat, hare populations in South Africa have undergone rapid population expansions in the Pleistocene.

The following specific research questions were formulated:

1) What is the amount of mitochondrial genetic variation within and among hare species in South Africa?
2) What is the amount of nuclear genetic variation within and among hare species in South Africa and how does it compare to the mtDNA variation?
3) Do the genetic data support the current species and subspecies taxonomy for hares in South Africa?
4) How is the genetic variation within species partitioned in relation to geography?
5) Which inferences with regards to current or historical evolutionary processes can phylogeographic analyses draw from the genetic data?

The strategy pursued to answer these questions was as follows:

- Collect hare specimens from all over South Africa and neighboring countries
- Determine mtDNA CR-I sequences for all collected hare specimens in order to investigate the population genetic structuring within *L. saxatilis* and *L. capensis* (chapters 4 and 6)
- Determine mitochondrial cytochrome *b* sequences for all collected *Lepus* specimens in order to readdress the question of species and subspecies delimitations among South African hares (chapter 7)
- Develop a microsatellite library for *L. saxatilis* (chapters 2 and 3)
- Screen all sampled animals with the developed microsatellites for genetic population structure and possible species-diagnostic subdivisions (chapters 5 and 6)
- Assess the degree of concordance of mitochondrial and nuclear genetic structure in South African hares (chapters 5, 6, and 7)

Chapter 2

Isolation and characterization of six polymorphic microsatellite loci in the scrub hare (*Lepus saxatilis* F. Cuvier, 1823)

2.1 Abstract

We have developed six novel, polymorphic microsatellite markers for the scrub hare, *Lepus saxatilis*. These markers have been tested for 150 animals from 26 different localities across southern Africa. All six loci amplify consistently well and most are highly polymorphic. The microsatellites presented here will be useful for phylogeographic studies in the scrub hare and other species of the genus *Lepus*.

2.2 Primer Note

Prinsloo and Robinson (1992) and Matthee and Robinson (1996) found marked genetic breaks among populations of two rock-dwelling small mammals of South Africa, the rock hyrax (*Procavia capensis*) and Smith's red rock rabbit (*Pronolagus rupestris*). It would be interesting to see if a similar phylogeographic pattern is reflected in a taxon with a different habitat preference but whose range spans the breaks shown in the strictly rocky outcrop species. An ideal candidate would be the scrub hare (*Lepus saxatilis*), with the overall goal to gain more detailed insight into the region's biogeographic history. In order to address this question we isolated and characterized six microsatellite loci from the scrub hare.

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Total genomic DNA was extracted from muscle tissue of a single *L. saxatilis* specimen using the phenol-chloroform method (Sambrook *et al.* 1989). Isolation of microsatellites was performed following Hillis *et al.* (1996a). The extracted DNA was digested to completion with *Sau3*A1. Fragments between 300-900 basepairs (bp) long were selected, ligated to a *Bam*HI digested pBS vector, and cloned in *Escherichia coli* DH5α competent cells. A total of 1131 clones were screened with a γ–^{32}P-labelled $(GT)_{15}$ oligonucleotide probe yielding 26 positives. PCR amplification and sequencing using the ABI Big Dye™ terminator cycle sequencing protocol with M13 vector primers detected 22 sequences with a microsatellite insert. Primer pairs flanking the microsatellite repeats of 12 loci were designed using Primer 3 software (Rozen & Skaletsky 1998). In a set of "cold-tests" we successfully determined the optimal PCR conditions for the 12 primer pairs and amplified template DNA from 15 geographically divergent scrub hare specimens for each locus. PCR products were visualized on 10% denaturing polyacrylamide gels stained with SYBR GREEN I Nucleic Acid Gel Stain (Roche) and sized against a 100 bp DNA ladder (Promega). Four loci proved to be monomorphic. However, eight loci amplified consistently and were polymorphic for the test specimens. Fluorescently labeled forward-primers (PE, Applied Biosystems) were obtained for each of these eight polymorphic loci. In a new set of PCR optimizations only six of the eight labeled primer pairs amplified consistently (Table 2.1); two loci (Lsa5 and Lsa7) dropped out possibly due to light damage to the fluorescent labels. Amplifications were performed in a 10-μl final volume containing approximately 10 ng of DNA, 1-1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.35 units of Expand High Fidelity polymerase (Roche), 10x buffer, and 4 pmol of each primer. The cycling parameters, using an Eppendorf Mastercycler® gradient, were as follows: an initial denaturation step at 94 °C for 3 min was followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature and 30 s at 72 °C. For loci Lsa1 and 6 a final elongation step of 20 min at 72 °C was performed, while for loci Lsa2, 3, 4 and 8 this step was omitted due to the excessive production of the "plus-A-peak" making the scoring of alleles problematic. Amplified products were run on an ABI 377 automated sequencer and fragment length was assigned using the GENESCAN and GENOTYPER software packages with GENESCAN-500 Tamra (PE, Applied Biosystems) as the internal size standard.

We genotyped 150 scrub hares from 25 localities (compound genotypes in Appendix Table A9) and found that allelic diversity was high, ranging from four to nine alleles per locus (Table 2.2). Expected and observed heterozygosities and *P*-values for departure from Hardy-Weinberg equilibrium were calculated in POPGENE (Yeh *et al.* 1997). Observed heterozygosity (H_O) figures ranged from 0.22 to 0.74, indicating a high variability at each locus. Lsa3 and Lsa4 were not in Hardy-Weinberg equilibrium (heterozygote deficiency), possibly due to scoring errors, null alleles or substructured populations (Jin & Chakraborty 1995). Given that each locus is characterized by an average of five alleles this set of six microsatellites will be useful for assessing the nuclear genetic structure within and among natural populations of scrub hares.

Table 2.1 Microsatellite motifs, annealing temperatures, $MgCl_2$ concentrations, primer sequences, and fluorescent labels.

Locus	Repeat array in cloned allele	GenBank acc. no	$T_a/MgCl_2$	Primer sequence (5' > 3')	Label
Lsa1	$(CA)_9$ CT $(CA)_2$ = imp. $(CA)_{12}$	AF491762	53 °C/1.5mM	F: CCT TGC AGG TTT TCA GCC TC R: GCT GTA GAA AAT GAG AGG GAC	FAM
Lsa2	$(AC)_{13}$	AF491763	57 °C/1.5mM	F: GGT ACT CTA TTA GGG AAC CCG R: GCT AGT TGC CAT TAG CTC CC	HEX
Lsa3	$(AC)_{10}$	AF491764	56 °C/1mM	F: TGC CTT AAA GGC TGA TTC TG R: GGA TAT CAA AGA ACA TGC CC	TET
Lsa4	CA GA $(CA)_8$ = imp. $(CA)_{10}$	AF491769	50 °C/1.5mM	F: CTG AGT GTA GTA TTT TCA GG R: GTG CCA TAT TCA TCT AAT GG	HEX
Lsa5	$(GT)_8$ $(GAAAGA)_2$ $(GA)_{13}$	AF491765	60 °C/1 mM	F: GGA AAT AAT GCT TGA GGC AGG G R: GGT CCC CTG CAC CCA TGT GG	FAM
Lsa6	$(AC)_{10}$	AF491766	53 °C/1.5mM	F: CCT AAG ATG AAA TGG ATA AGT T R: CTC TTC TGT TTT CTG GAG CA	TET
Lsa7	$(GA)_5$ AA $(GA)_8$	AF491767	55 °C/1 mM	F: CCA GCC CTG AAC ATT GTA AGC R: GCA GCG GCC ATT GGA GGG T	HEX
Lsa8	$(GT)_{11}$	AF491768	53 °C/1.5mM	F: AAG GTA TTA AAT TGG GCA CTC R: GAC TGA AAT TGA TGT GCT ACC	FAM

Table 2.2 Scoring results for *Lepus saxatilis*: k = number of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity, *P* = probability values of rejecting Hardy-Weinberg expectations (likelihood ratio test), the bold values indicate significant departure.

Locus	Size-range (bp)	k	H_E	H_O	*P*
Lsa1	162 - 174	7	0.69	0.74	0.356
Lsa2	235 - 255	9	0.59	0.56	0.195
Lsa3	198 – 212	7	0.68	0.50	**0.000**
Lsa4	107 – 113	4	0.26	0.20	**0.001**
Lsa6	166 - 178	6	0.24	0.22	0.129
Lsa8	180 - 194	7	0.66	0.68	0.543

Chapter 3

Cross-species and cross-genera amplification of polymorphic microsatellite loci developed for the scrub hare, *Lepus saxatilis* (Leporidae, Lagomorpha)

3.1 Abstract

The species and subspecies delimitations within many leporids (hares and rabbits) are controversial. In order to decide on appropriate management strategies for endangered taxa it will be necessary to identify conservation relevant units. To address this goal we tested six microsatellite loci originally developed for the scrub hare (*Lepus saxatilis*) for cross-species and cross-genus amplification. Four of the six loci amplified consistently in five different genera.

3.2 Primer Note

Leporidae (hares and rabbits) play an important role in many ecosystems, as they are a major prey item of many predators. In order to establish conservation priorities the IUCN lagomorph specialist group (Flux and Angermann 1990) has called for molecular genetic studies to clarify the still confused taxonomy within the genus *Lepus* (true hares). For South Africa it is a matter of controversy as to how many (two or three) and which species of hares occur.

i 1622324x
b15667182

Kryger *et al.* (in press; chapter 2) have developed six polymorphic microsatellite markers for the scrub hare (*L. saxatilis*). Surridge *et al.* (1997) reported on the successful cross-genera amplification of microsatellite markers developed for the European rabbit (*Oryctolagus cuniculus*) in other lagomorphs. Here we test these markers for their utility in related leporid species from five different genera.

Amplifications and Genescans were performed as described in Kryger *et al.* (in press; chapter 2). We successfully genotyped 67 specimens of *L. capensis* (Cape hare) with all six loci (for compound genotypes see Appendix Table A9). All loci were highly polymorphic in the Cape hare with moderate to high observed heterozygosities as calculated by POPGENE (Yeh *et al.* 1997); allelic diversity ranged from 3-12 alleles per locus (Table 3.1). Departures from Hardy-Weinberg expectations were only observed in Lsa1. This is probably due to scoring errors or the presence of null alleles.

Further cross-species amplifications (Table 3.2) were conducted for all loci in *Pronolagus rupestris* (Smith's red rock rabbit; n = 2), *Pronolagus crassicaudatus* (Natal red rock rabbit; n = 2), and *Bunolagus monticularis* (Riverine rabbit, one of the most endangered mammals in Africa; n = 2). All loci with the exception of Lsa3 amplified in the single *Caprolagus hispidus* (Hispid hare) specimen available to us. PCR products for three of the six loci were observed in two *P. randensis* (Jameson's red rock rabbit) and one *Oryctolagus cuniculus* (European rabbit) specimen.

Four loci (Lsa1, 3, 6, and 8) are polymorphic in all cross-genera amplifications, despite the small sample sizes. This result underscores their potential usefulness in taxonomic and population studies in the tested taxa. Additionally, they will be of value in the management of commercially important species such as the European rabbit and in determining effective population size and identifying conservation units *sensu* Moritz (1994b) in endangered species. Given that the primers amplified homologous loci in the European rabbit, the repeat-array flanking regions may have been conserved for over 12-16 million years (date for most recent common ancestor of *Oryctolagus* and *Lepus*; Halanych & Robinson 1999). Thus these markers will potentially be useful in conservation genetic studies of other endangered members of the genus *Lepus* and perhaps also other genera such as *Sylvilagus, Romerolagus, and Pentalagus*.

A comparison of the number of private alleles per locus resulted in nine Lsa2 alleles in the scrub hare, but only three in the Cape hare and one in the remaining taxa. The detection of six diagnostic alleles in the southern African *Lepus* is important since the phenotypic distinction between *L. saxatilis* and *L. capensis* in some areas of sympatry is problematic. Likewise, Lsa3 produced 12 alleles in *L. capensis* in comparison to seven in *L. saxatilis* and two in all other genera. Allele 164 from Lsa6 and allele 178 from Lsa8 appear to be diagnostic for the genus *Pronolagus*.

Table 3.1 Scoring results for *Lepus capensis*: k = number of alleles, n = sample size, H_E = expected heterozygosity, H_O = observed heterozygosity, *P* = probability values of rejecting the Hardy-Weinberg expectations (likelihood ratio test), the bold value indicates significant departure.

Locus	Size-range (bp) in L. saxatilis	Size-range (bp) in L. capensis	k	H_E	H_O	*P*
Lsa1	162 - 174	162 – 174	7	0.75	0.73	**0.001**
Lsa2	235 - 255	245 - 249	3	0.14	0.10	0.208
Lsa3	198 - 212	200 - 226	12	0.67	0.35	0.077
Lsa4	107 - 113	107 - 115	5	0.49	0.43	0.400
Lsa6	166 - 178	170 - 176	4	0.56	0.54	0.117
Lsa8	180 - 194	180 - 196	9	0.74	0.77	0.990

Table 3.2 Cross-genera-amplification success; species, n = number of animals sampled; number of different alleles found/allelic size range for each locus; * = no amplification

Species (n)	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
Bunolagus monticularis Riverine rabbit (2)	2/166 - 170	1/249	2/200 - 204	1/107	2/170 - 172	3/184 - 188
Pronolagus rupestris Smith's red rock rabbit (3)	1/166	1/249	2/200 - 204	1/107	3/164 - 172	3/182 - 188
Pronolagus randensis (2) Jameson's red rock rabbit	1/170	*	*	1/107	3/164 - 172	2/178 - 182
Pronolagus crassicaudatus Greater red rock rabbit (2)	4/166 - 172	1/249	2/200 - 204	1/107	2/170 - 172	3/178 - 188
Pronolagus spec. (2) Red rock rabbit	2/166 - 170	1/249	2/200 - 204	1/107	2/170 - 172	3/178 - 188
Oryctolagus cuniculus European rabbit (1)	2/166 - 168	*	*	1/107	1/170	2/186 - 188
Caprolagus hispidus Hispid hare (1)	1/166	1/249	*	1/107	1/170	2/186 - 188

Chapter 4

Mitochondrial DNA population structure and population history of the Scrub Hare, *Lepus saxatilis*, in southern Africa

4.1 Abstract

The genetic differentiation among populations of the South African scrub hare (*Lepus saxatilis*) was examined using hypervariable mitochondrial DNA (mtDNA) control region I sequences. Neighbor-joining analysis revealed a phylogeographic pattern that did not correspond to the current subspecies delineations. The CR-I sequence data delimit scrub hares into three major maternal lineages. The three phylogenetic assemblages exhibited different geographical distributions. AMOVA analyses and exact tests for population differentiation confirmed this phylogeographic partitioning. One lineage (SW) was confined to the southwestern Cape, the second lineage (N) was exclusively found in the northern part of South Africa and in the neighbouring countries, and the third lineage (C) was predominant in the central parts of South Africa. This spatial distribution did not coincide with the ranges of the ten described subspecies covered by our sampling regime. The lineages C and N overlapped in an area including eastern parts of South Africa and southern Namibia. The presence of both lineages in that area of overlap was interpreted as the result of secondary contact due to recent range expansions after the two lineages had undergone a population restriction approximately 18 000 years ago. Analyses of contemporary gene flow disclosed an exchange of migrants between N and C, which was biased towards a movement from C to N. The SW group represents a very distinct evolutionary lineage that has been isolated for more than 45 000 years. It does not exchange female migrants with the other two groups. This finding could carry conservation implications. Mismatch distribution analyses indicated sudden population size expansions in the history of all three populations.

4.2 Introduction

The scrub hare (*Lepus saxatilis*) is a member of the family Leporidae in the mammalian order Lagomorpha (Meesters *et al.* 1986). The species is widespread throughout the southern African subcontinent (Roberts 1951; Skinner & Smithers 1990) and prefers scrub or savanna woodland habitat with grass cover but easily takes to agriculturally developed areas. The life history of the scrub hare is characterized by a high reproductive rate and fast population turnover. Hares are renowned for their dispersal capabilities and single individuals as well as whole populations can move over vast distances (Schneider 1990). Home range sizes usually vary according to habitat and season (Hulbert *et al.* 1996) and females seem more sedentary than males (Reitz & Leonard 1994).

The subspecies taxonomy for the scrub hare has mainly been derived from characters such as body size, fur coloration, and geographic locality (Roberts 1951). Local differences in these traits are a common phenomenon in South African small mammals and seem more a consequence of climate and habitat than genotype (Roberts 1951). Avise *et al.* (1987) strongly opposed subspecies delineations based on traits whose genetic underpinnings are unknown while, Zink & Avise (1990) cautioned that intraspecific taxonomies based on morphological characters that exhibit phenotypic plasticity tend to reveal ecological associations rather than evolutionary relationships. Robinson & Dippenaar (1987) reported on a cline in body size and skull and ear length in the scrub hare from the southwestern Cape Province to Zimbabwe, leading Skinner & Smithers (1990) to question the validity of the subspecies taxonomy. To date, there has been no genetic assessment of the soundness or otherwise of the subspecies designations within *L. saxatilis*.

Little is known about the evolutionary history of South African hares in general. The fossil record suggests the appearance of the genus on the continent in the late Pliocene or early Pleistocene approximately 4-1.5 million years ago (Lavocat 1978; Carroll 1988). At this time, the African climate was characterized by cycles of cold and dry phases alternating with warm and humid phases (Grubb 1978; DeMenocal 1995). During the dry episodes, savanna habitats extended in range and humid forests contracted with the reverse occurring during moist episodes (Coe & Skinner 1993). These oscillating habitat

expansions and contractions during the Pleistocene profoundly influenced the evolution of African mammals and are assumed to have induced diversification events at the species and subspecies level in many taxa (Ewer & Cooke 1964; Grant & Leslie 1993). The legacy of these past climatic events may often be reflected in geographic partitioning of genetic variation, which allows for the reconstruction of evolutionary history.

In this study we investigated the amount and geographical structuring of mtDNA variation of the South African scrub hares using rapidly evolving CR-I sequences. The main objectives were (i) to assess whether the mtDNA genetic variation in *L. saxatilis* is geographically structured, (ii) to elucidate the population history of the scrub hare in southern Africa, (iii) to determine if any of the scrub hare subspecies represent distinct evolutionary lineages.

4.3 Materials and methods

Data collection

Samples and DNA extraction. Tissue samples from 159 scrub hares were obtained in 1999 and 2000. Four animals were collected in Namibia, ten in Zimbabwe, and one in Botswana; the rest of 144 hares were sampled from 25 different localities throughout South Africa (Fig. 4.1; Table 4.1).

Fresh muscle and heart tissue was transferred into a 20% DMSO/saturated salt solution (Amos & Hoelzel 1991) and stored at room temperature. Total genomic DNA was prepared by proteinase K-phenol-chloroform extraction followed by ethanol precipitation (Sambrook *et al.* 1989).

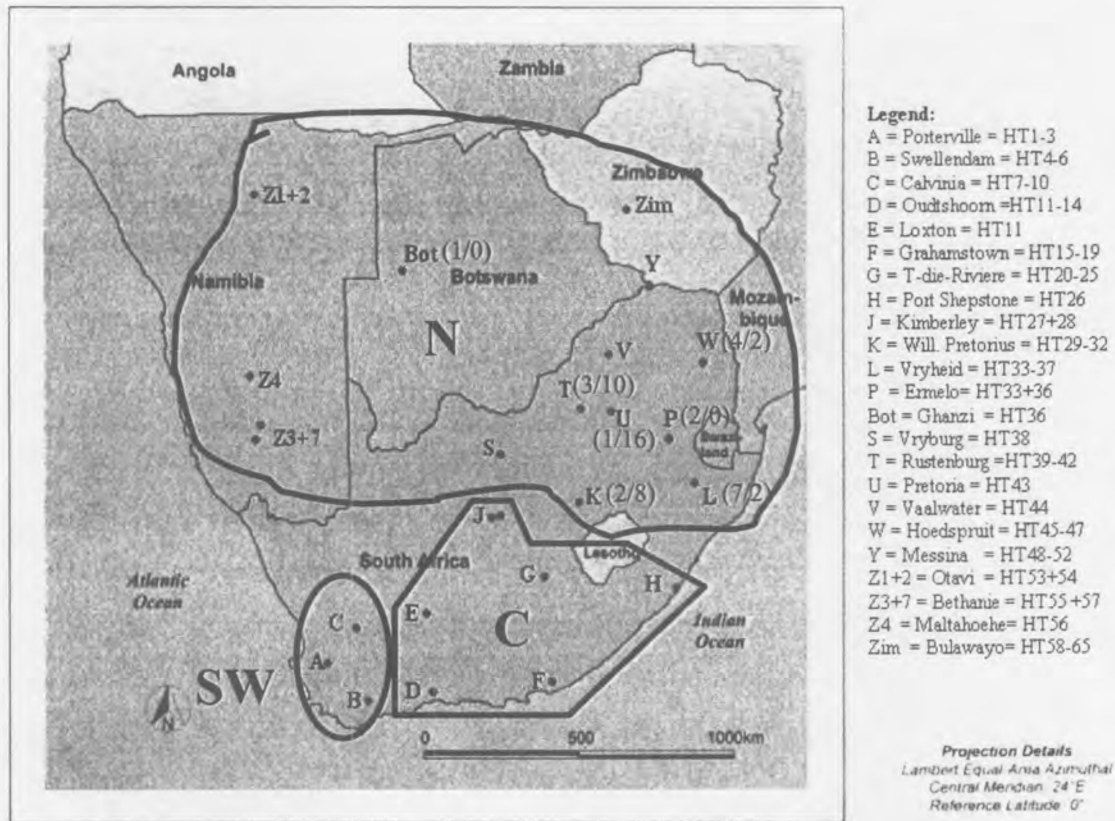

Fig. 4.1 Collection localities and corresponding mtDNA haplotypes of *Lepus saxatilis*. See Table 4.1 for subspecies designations and number of individuals per haplotype. The encircled areas signify the three phylogeographic assemblages (SW, C, and N) identified via NJ analysis. The numbers following Bot, K, L, P, T, U & W indicate which proportion of the animals sampled at that particular location clustered in the central/in the northern assemblage (cf. Results).

Choice of genetic marker. Given previous reports of mtDNA heteroplasmy in lagomorphs (Mignotte *et al.* 1990; Biju-Duval *et al.* 1991; Casane *et al.* 1997) and its association with short and long tandem repeats towards the 3' end of the control region (CR), we amplified the complete fragment. This was done using a *Lepus*-specific primer LsL1 (5' AGC ACC CAA AGC TGA AAT 3' - the 3' terminal nucleotide of this primer binds to position 15 427 of the rabbit mitochondrial genome; see Gissi *et al.* 1998) and a modified version of 559 which we called H79 (5' CAA TAR AAA GGC CAG GAC C 3' - the 3' terminal nucleotide of this primer binds to position 79 in the 5' end of the 12Sr RNA). Sequencing with specifically designed internal primers LsL3 (5' CCT CCG - TGA AAC CAG CAA CCC G 3'- annealing at position 15 869) and LsL4 (5' CCT CAT-

AAT TAT GAG CCG GG 3' - annealing at position 16 188) and 559 confirmed the presence of a short and a long tandem repeat in the 3' domain of the scrub hare control region. We detected a 10 bp motif (GenBank Acc no AY 092772), starting at position 16 220, that was tandemly repeated at least 30 times, and a 142 bp long motif (GenBank Acc no AY 092773), starting at position 16 559, that was repeated at least four times. Repeat arrays like these are problematic for inferring phylogenetic associations due to inconsistent amplification during PCR (Lunt *et al.* 1998), the formation of secondary structures and related substitutional biases (Stewart & Baker 1994), and the violation of the assumption of neutral evolution (Casane *et al.* 1997). We therefore decided to exclude the right and conserved central domain of the control region from our investigations. Lopez *et al.* (1997) found that the 5' end of the CR is generally one of the most divergent regions of the mtDNA. Consequently, this hypervariable CR-I has extensively been used as a population marker (Eizirik *et al.* 1998; Fry & Zink 1998; Simonsen *et al.* 1998; Stepien & Faber 1998; Matsuhashi *et al.* 1999; Nagata *et al.* 1999; Nyakaana & Arctander 1999; Houlden *et al.* 1999; Pierpaoli *et al.* 1999; Birungi & Arctander 2000; Pope *et al.* 2000; Davison *et al.* 2001; Grau Nersting & Arctander 2001), justifying its use in this investigation.

Control region amplification and sequencing. A 438 bp long fragment of the 5' end of the mtDNA CR was amplified using the two *Lepus*-specific primers LsL1 and LsH2 (5' CGG GTT GCT GGT TTC ACG G 3', annealing at position 15 847). The amplified region started in the tRNA Proline and spanned the entire left domain of the control region (CR-I). It terminated just before the central conserved region (Taberlet 1996). Polymerase chain reaction (PCR; Saiki *et al.* 1988; White *et al.* 1989) amplifications were performed in a 50 µl total reaction volume containing approximately 40 ng of the DNA, 250 µM dNTPs, 1 mM $MgCl_2$, 1 µM of each of the two above listed primers and one unit of Super-Therm DNA polymerase (Southern Cross Biotech).

Amplification parameters were as follows: one initial denaturing cycle for 5 min at 94 °C; 30 cycles of 30 sec denaturing at 94 °C, 30 sec annealing at 58 °C and 1 min elongation at 72 °C; a final elongation step of 7 min at 72 °C. Each amplification experiment included a negative control to check for contamination. Amplifications

resulted in single, well-defined bands as visualized via agarose gel electrophoresis. The absence of ambiguous or double bands confirmed the absence of heteroplasmy.

Primers were removed from the amplification with the High Pure PCR product purification kit (Boehringer Mannheim). The amplified fragments were cycle sequenced in both directions (with LsL1 and LsH2) using the Big Dye Terminator Cycle Sequencing Ready Reaction kit and electrophoresed on an ABI 377 PRISM automated sequencer (PE, Applied Biosystems).

Data analysis

Phylogenetic analysis. The corresponding L- and H-primer sequences of each individual were aligned, compared and edited in SEQUENCE NAVIGATOR (PE, Applied Biosystems). The consensus sequences of all individuals were then automatically aligned with the program CLUSTAL X (Thompson *et al.* 1997) and these alignments were manually optimized in order to eliminate excess gaps. The sequences were deposited in GenBank (Accession nos AF491386 - AF491450).

Analyses to retrieve phylogenetic associations among lineages were performed in PAUP*4.0 beta 10 version (Swofford 2002). Due to the difficulty of defining an appropriate outgroup for an intraspecific study (Maddison *et al.* 1992; Crandall & Templeton 1993; Castello and Templeton 1994; Pierpaoli *et al.* 1999), the midpoint rooting option was applied (Swofford 2002). We ran a maximum likelihood ratio test as implemented in MODELTEST Version 3.04 (Posada & Crandall 1998) in order to determine the model of nucleotide substitution that fits the presented data most adequately. With a statistical significance of $P = 0.01$ the HKY85 model (Hasegawa *et al.* 1985) with gamma correction (Gu & Zhang 1997) obtained the best likelihood score and was thus selected for the neighbor-joining (NJ) analysis. The HKY-85 Γ model of sequence evolution accounts for differences in transition/transversion ratios (Ti/Tv), unequal base frequencies, and among site rate heterogeneity (Yang *et al.* 1994; Yang 1996; Yang & Kumar 1996). The Ti/Tv ratio and the shape parameter (α) for the gamma distribution were estimated using the maximum likelihood (ML) method in PAUP. A gene tree containing all the haplotypes was constructed with the NJ algorithm (Saitou & Nei

1987) based on pairwise distances and using the option of randomized tie breaking. Nodal support for the tree was assessed by 1000 bootstrap replicates (Felsenstein 1985; Hillis & Bull 1993) and changing to outgroup-rooting against the most divergent haplotype.

We performed 100 heuristic searches for the parsimony analysis (Felsenstein 1982, 1988), obtaining the starting trees by random stepwise addition and swapping the branches via the tree-bisection-reconnection function. Alignment gaps were treated as a fifth character state and coded following Simmons & Ochoterena (2000) in order to account for the insertions/deletions present among members of the ingroup taxa.

Polymorphism and partitioning of genetic variation. The hierarchical components of the mtDNA variation were computed under the AMOVA (Analysis of Molecular Variance) framework (Weir & Cockerham 1984; Excoffier *et al.* 1992) using the ARLEQUIN software package Version 2.000 by Schneider *et al.* (2000, http://anthropologie.unige.ch/arlequin). The AMOVA procedure produces Φ-statistics analogous to Wright's (1951) *F*-statistics and incorporates both the estimated divergences between sequences and their frequencies. The significance of these Φ-statistics was tested using 10 000 randomized permutations. In addition, we applied the exact test for population differentiation as described by Raymond & Rousset (1995b) and Goudet *et al.* (1996) based on a Markov Chain procedure with 10 000 steps per Markov Chain and 1000 dememorization steps. We also calculated pairwise F_{ST} values for all pairs of populations and the interpopulational divergence times allowing for unequal population sizes following Gaggiotti & Excoffier (2000). Furthermore, we computed the average pairwise intrapopulational differences ($\pi_X = D_X$), the average interpopulational differences (π_{XY} = Nei's raw D_{XY}), and the corrected average interpopulational differences (($\pi_X + \pi_Y)/2$ = Nei's net D_A) according to Nei & Li (1979) in ARLEQUIN. The program DNASP Version 3.53 (Rozas & Rozas 1999) was employed to calculate haplotype diversity (H), the average number of pairwise nucleotide differences (k; Tajima 1983), and the nucleotide diversity (π; Nei 1987).

Mutation Rate and Coalescence. We calculated the mutation rate and the coalescence time (= time to the most recent common ancestor, TMRCA) of the CR-I haplotypes of the scrub hare following Rooney *et al.* (2001). The number of nucleotide substitutions per site (d) were estimated by comparing the ingroup with an outgroup species, the Cape hare (*L. capensis*), using the formula $d = (\mathrm{T}v + \mathrm{T}v\mathrm{R})/m$, where T$v$ is the number of transversions between the focal species and the outgroup species, R is the ratio of transitions to transversions within the ingroup, and m is the length of the investigated DNA fragment (420bp in our case). The rate of nucleotide substitution per site per lineage per year (λ) was estimated using $\lambda = d/2\mathrm{T}$, where T stands for the divergence time of the two compared species. We obtained the mutation rate per nucleotide site per generation (μ) by solving the equation $\mu = \lambda t_g$, where t_g is the generation time in years. The mutation rate per haplotype (v) was calculated by $v = m\mu$. Finally, the coalescence time in generations was calculated using $t = \tau/2v$ (Rogers & Harpending 1992) and the TMRCA in years was estimated by multiplying t with the generation time.

For populations at genetic/demographic equilibrium the observed nucleotide diversity corresponds to the mutation parameter ($\theta = 2N_e\mu$) if the nucleotide fragment under investigation is not selected for (Nei 1987). We tested for the assumption of neutrality of the analyzed CR-I sequences by computing Fu and Li's D*, Fu and Li's F*, and Tajima's D statistics (Fu & Li 1993; Tajima 1989a) as implemented in DNASP. Coalescent theory based maximum likelihood estimates for migration rates (Beerli & Felsenstein 2001) among populations were calculated in the program MIGRATE Version 1.1 (Beerli 1997-2001) which applies a Markov chain Monte Carlo approach. The MIGRATE estimates for theta (per site) were interpreted as indicators of the extant effective population size by solving the above equation to $N_e = \theta/2\mu$, with μ as the mutation rate per site per generation.

Mismatch distributions. Mismatch distribution analyses were performed under the sudden expansion model proposed by Rogers & Harpending (1992) as implemented in ARLEQUIN. The observed distribution of pairwise genetic differences between haplotypes within a population allows distinction between different demographic histories. Stable or slowly declining populations at demographic equilibrium generate a multimodal

distribution reflecting the stochastic shape of the underlying gene trees. Populations that went through a recent sudden bottleneck or expansion produce a unimodal distribution (Rogers & Harpending 1992). When a stationary haploid population at equilibrium suddenly expands from a population size N_0 to a population size N_1, the following formula from Li (1977) describes the probability of observing S differences between two randomly chosen haplotypes:

$$F_S(\tau, \theta_0, \theta_1) = F_S(\theta_1) + \exp(-\tau((\theta_1 + 1)/\theta_1))) \sum_{j=0}^{S} \tau^j/j! \; [F_{S-j}(\theta_0) - F_{S-j}(\theta_1)].$$

According to Watterson (1975), the probability of observing two haplotypes with S differences in a stationary population can be expressed by:

$$F_S(\theta) = \theta^S/(\theta + 1)^{S+1},$$

the mutation parameter of the initial population being $\theta_0 = 2\nu N_0$ and that of the final population $\theta_1 = 2\nu N_1$, with $\tau = 2\nu t$; N is the effective maternal population size, τ is the moment estimator to the expansion time in generations, and ν is the mutation rate per generation for the complete haplotype.

We estimated θ_0, and θ_1 in three different ways. First, as implemented in DNASP Version 3.53 (Rozas & Rozas 1999) using the number of segregating sites and assuming a stable population size and genetic equilibrium with the resulting values describing θ per gene (Watterson 1975). Secondly, as implemented in FLUCTUATE Version 1.3 (Kuhner *et al.* 1998) using the genealogical relationships among the observed haplotypes, and allowing for historical fluctuations of population sizes by the coalescent Metropolis-Hastings Markov Chain (MHMC) method, resulting in maximum likelihood estimates of θ per site (Kuhner *et al.* 1995, 1998). And thirdly, as implemented in ARLEQUIN using the general non-linear least-square (N-L L-S) approach proposed by Schneider & Excoffier (1999) which obtains approximate confidence intervals for the two parameters and for the expansion time (τ) by parametric bootstrapping and calculates a goodness-of-fit test

based on the sum of squared differences and Harpending's raggedness index (Harpending 1994).

4.4 Results

Scrub hare mtDNA CR-I

The electropherograms were clear and showed no ambiguous peaks (and therefore no heteroplasmy) throughout the sequenced product. Only the extreme ends of the 438 bp long fragment broke up in quality and were therefore excluded from the further analyses. The 420 bp fragment of mitochondrial CR-I included in the analyses comprised 83 variable sites (19.76%), 49 (11.67%) of which were parsimony informative, and 2 of which were indels (position 45 & 46). The Ti/Tv ratio was relatively high (11), but well within the range of values for other vertebrate CRs. Pierpaoli *et al.* (1999), Matthee & Robinson (1999), and Eizirik *et al.* (2001) reported CR Ti/Tv ratios of 3.9 among hare species, 18 in the sable antelope, and 6 in the jaguar, respectively. The high Ti/Tv ratio together with the high intraspecific variation (see below) conformed closely to the expected characteristics of mtDNA CRs. We therefore considered it highly unlikely that we had amplified any nuclear paralogues (Lopez *et al.* 1994; Zhang & Hewitt 1996). Little heterogeneity in substitution rates among nucleotide sites was observed (α = 0.9463). The nucleotide composition was characterized by a scarcity of G (only 9% versus approximately 30% for each of the other nucleotides), which is typical for vertebrate control regions (Baker & Marshall 1997).

Distribution and Relationships of CR-I haplotypes. The sequenced CR-I fragment defined 65 unique mitochondrial haplotypes among the 159 investigated individuals (Tables 4.1 and 4.2; for complete alignment see Appendix Table A2). Accordingly, haplotype diversity expressed over the complete sample was quite high (H = 0.97; Table 4.3). Most sample localities were characterized by a set of private haplotypes and 27 haplotypes were singletons (Table 4.1).

Table 4.1 Sampling locations (in bold), haplotype designations (HT), and field numbers of the animals genotyped in this study; Rep = representative. Subspecies designations are according to Roberts (1951) and assemblages as resulting from NJ analysis (Fig. 4.2).

Assemblage	Subspecies	Population	HT	Rep	Sharing same haplotype
Southwest	*L .s .saxatilis*	**A – Porterville**	1	A1	
			2	A2	A3, A5, A7
			3	A4	A6
	L. s. saxatilis	**B - Swellendam**	4	B1	B6, B9, B10, D5 (Oudtshoorn)
			5	B2	B4, B5, B7, B8
			6	B3	
	L. s. saxatilis	**C - Calvinia**	7	C1	C3, C7, C9, C10
			8	C2	
			9	C4	C8
			10	C5	C6
Central	*L. s. saxatilis*	**D – Oudtshoorn**	11	D1	D6, D7, D8, D9, D10, E1-E5 (**E – Loxton** – *L. s. megalotis*)
			12	D2	F1, F3
			13	D3	
			14	D4	
	L. s. albaniensis	**F – Grahamstown**	15	F2	F4
			16	F5	F8
			17	F6	F9
			18	F7	
			19	F10	F11 (Kirkwood)
	L. s. chiversi	**G – Tussen-di-Rivieren Game Reserve**	20	G1	G5 K2
			21	G2	
			22	G3	
			23	G4	G8, G9
			24	G6	G7
			25	G10	
	?	**H – Port Shepstone**	26	H1	H2, H3
	L. s. orangiensis	**J – Kimberley**	27	J6	J10
			28	J7	
North	*L. s. orangiensis*	**K – Willem Pretorius Game Reserve**	29	K1	K4 , K9
			30	K3	K7, K10
			31	K5	K8 T3, T5, T6, T11 (Rustenburg/Pilanesberg) U4 & U5, U10 (Pretoria/Pienaarsrivier) V1, V5 & V6 (Vaalwater/Thabazimbi) Y1& Y7 (Messina)
Central			32	K6	

Assemblage	Subspecies	Population	HT	Rep	Sharing same haplotype
Central	*L. s. zuluensis*	**L - Vryheid**	33	L1	L3, L4, L8, P13 (P – Ermelo - *L. s. zuluensis*)
North			34	L2	
Central			35	L5	L7
Central			36	L6	P4 (P - Ermelo) Bot (Bot – Botswana *L. s. bechuane*)
North			37	L9	
	L. s. subrufus	**S - Vryburg**	38	S1	V2 & V4 (Vaalwater/Thabazimbi)
Central	*L. s. subrufus*	**T – Rustenburg/ Pilanesberg**	39	T1	T10 & T13 U8 (Pretoria/Pienaarsrivier)
North			40	T2	T4 & T7 U1, U2, U3, U9, U11, U12, U13, U14, U15, U16, U17 (Pretoria/Pienaarsriv.)
			41	T8	T9
			42	T12	Y4 (Messina)
	L. s. subrufus	**U – Pretoria/Pienaarsrivier**	43	U6	U7
	L. s. zuluensis	**V – Vaalwater/Thabazimbi**	44	V3	
Central	*L. s. zuluensis*	**W – Hoedspruit**	45	W1	W2
Central			46	W3	W4
North			47	W5	W6
	L. s. zuluensis	**Y – Messina**	48	Y2	Y8
			49	Y3	Y5
			50	Y6	
			51	Y9	
			52	Y10	
	L. s. herero	**Z - Namibia**	53	Z1	
			54	Z2	
			55	Z3	
			56	Z4	
			57	Z7	
	L. s. micklemi	**Zim – Zimbabwe**	58	Zim 1	Zim9
			59	Zim 2	
			60	Zim 3	
			61	Zim 4	
			62	Zim 5	
			63	Zim 6	Zim7
			64	Zim 8	
			65	Zim 10	

Table 4.2 Variable sites defining 65 CR-I haplotypes for 159 individuals in *Lepus saxatilis*. For geographic locality and number of individuals carrying a specific haplotype cf. Fig.4.1 and Table 4.1.

```
                       1111111111111111111122222222222222222222222222222222222222333333333334
            111244477990000111112233455689901111222222333344444455555666677778899011224666772
          37135256769380239012340906709420211278123569068904567801356023501583429545784239450
-------------------------------------------------------------------------------------------------
HT01      CTCTTA--CATCTTTACTATTTTAGCTGTTTTCCGCTTCTGTTTTATCCTCAACACCAACATTTATCATCCTATCACCTCGGT
HT02      ............................C....T.T.C....CC.....................CT..........TC....
HT03      .................C..........C....T.T......CC..C...................T...........C....
HT04      ........T...................C....T.........C.......................................
HT05      ........T..................AC....T.........C.......................................
HT06      ......A.T...................C....T.........C.........................T.............
HT07      ............................C....T.T......CC..................................C....
HT08      ..........................C.C....T.T......CC..................................C....
HT09      ............................C....T........CC......................T...........C....
HT10      ................T..........AC....T.T.......C.........T........................C....
HT11      ......A...C.C...T..C.C...GC.C....TA..C..AC.C..C.T.T.....TG.T.C..T.....T....G.......
HT12      ......A...C.C...T..C.C...GC.C....TA..C..AC.C....T.T.....TG.T.C..T.....T....G.......
HT13      ......A...C.C..GT..C.C...GC.C....TA..C..AC.C..C.T.T.....TG.T.C..T.....T....G.......
HT14      ......A...C.C..GT..C.C...GC.C....TA..CT.AC......T.T.....TG.T.C..T.....T....G.......
HT15      .........GC.C...T..C.C...GC.C....TA..C..AC.C....T.TG....TG.T.C..T.....T..C.G.......
HT16      ......A...C.C...T..C.C...GC.C....TA..C..AC.C....T.TG....TG.T.C..T.....T....G.......
HT17      ......A...C.C...T..C.C...GC.C....TA..C..AC.C....T...G...TG.T.C..T.....T....G.......
HT18      ......A.....C...T..C.C...GC.C....TA..C..AC.C....T.TG....T..T.C..T.....T....G.......
HT19      ......A...C.C...T..C.C...GC.C....TA..C..AC.C....T.TG....T..T.C..T.....T....G.......
HT20      ..........C.....T....CC..GC.C.....A..C..ACCC.......G....TG.T.CCCT....TT.......C....
HT21      ......A...C.C...T..C.....GC.C....TAT.C..AC.C....T.T.....TG.T....T.....T....G.......
HT22      ......A.....C...T.........CA.....TA..CT.A..CC.CT........T..TTC.CT..G.TTCG.....C.R..
HT23      ......A....TC...T....C...GCAC....T...CT.AC.C.............G.T.CC.T...............A..
HT24      ......A....TC...TC...C...GCAC.C..T...CT.AC.C..C.........TG.T.CCCCC............C.A..
HT25      ..........C.....T....CCG.GC.C.....A..C..ACCC.......G....TG.T.CCCT....TT.......C....
HT26      ..T...A.........T....C...GCAC....T....T.AC.C.........T..TG.T.CCCT.............C.A..
HT27      ..........C.....T....CC..GC.C.....A..C..ACCCC......G....TG.T.CCCT....TT.......C....
HT28      ......A...C.C...T..C.C...GC.C....TA..C..AC.C....T.TG..G.TG.T....T.....T....G.......
HT29      T.....A.....C....C...C...GC.C....T...CT....C...............T....T.....T.G.....C....
HT30      T.....A.....C........C...GC.C....T...CT....C...............T..C.T.....T.G.....C....
HT31      T.....A.....C........C...GC.C....T...CT....C...............T....T.....T.G.....C....
HT32      ......AA....C...T........GCAC....T...CT.AC.C............TG.T.CCCT.............C....
HT33      ......A.....C...T....C...GC.C....T...CT.AC.CC.C.........T..TTC.CT..G.TTCR.....YYR.Y
HT34      T.T.......C.C........C...GC.C....TA..C.....C.............G.T.C..T.....TYR.....YYR.Y
HT35      ......AA...T....T....C...GCAC....T...CT.AC.C.............G.T.CCCT.....YYR.....YYR.Y
HT36      ......A.....C.C.T........GC.C....T...CT.AC.C............TG.T.CCCT.............C....
HT37      Y.T.......C.C........C...GC.C....TA..C.....C...............T.C..T.....T.G.....CTA.C
HT38      T.....A.....C........C...GC.C....T...CT....C...............T....T.....T.......C...Y
HT39      ......A....TC.C.T....C...GCAC....T...CT.ACCC..C.........TG.T.CCCC.............C.A.Y
HT40      T....G......C........C...GC.C....T...C.....C...........T...T.C..T.....T...........Y
HT41      T.....A.....C........C...GC.C....T...CT....C...............T....T.....T.G...T.C...Y
HT42      T.....A.....C........C...GC.C....T...CT....C...............T.C..T.....T.G.....C....
HT43      T.....A.....C........CC..GC.C....T...CT....C......T........T....T.....T.G.....C...Y
HT44      T.....A.....C........C...GC.C....T...CT....C...............T.C..T.....T.......C....
HT45      ......A.....C...T...C....GC.....TT...CT.AC.CC...........T..TT..CT..G.TTCG.....C....
HT46      Y.....A.....C...T....C.G.GC.C........CT.AC.CC.C.........T..TTC.CT..G.TTCG.....C....
HT47      T.....A.....C........C...GC.C....T...C.....C...............T....T.....T.G.....C....
HT48      T...........C........C...GC.C....T...CT....C....T..........T....T.....T.G.....C...C
HT49      T.........C.C........CC..GC.C....T...C.....C...............T.C..T.....T.G.........Y
HT50      T.....A.....C........C...GC.C....T...CT....C...............T....T.....T.G..........
HT51      T...........C.C...G..C...GC.C....TA..CT..C.C...............T.C..T.....T.G.....C....
HT52      T...........C........C...GC.C....TA..CT...CC..C............T..C.T.....T.G.....C...C
HT53      T.........C.C........CC..GC.C....TA..C.....C...............T....T.....T.G.........C
HT54      T.........C.C........C...GC.C....TA..C.....C...............T....T.....T.G.........Y
HT55      ....C.....C.C...TC...C...GC.C.C..T.T.C...C.CC..............T..C.T.....T.......C.A..
HT56      T...C.....C.C...TC...C...GC.C.C..T.T.C...C.CC..............T..C.T.....T.......C.A..
HT57      TC.C........C..........GAGC.CA.AATA.CC.C..CC.TC....T..................TC......C.AA.
HT58      T...C.A...C.C....C...C...AC.C....T...C...C.C..C.T.........GT.C..T.....T.......C....
HT59      T.....A.....C....C...C...GC.C....T...C...C.C..C.TCT........T....T...C.T...T...C...A
HT60      T.....A...C.C....C...C...GC.C....T...C...C.C..C.T..........T....T...C.T.......C...C
HT61      T.........C.C........C...GC.C....TA..CT..C.C...............T....T.....T.G.....C...C
HT62      T.....AA..C.C....C...C...GC.C..C.T...C...C.C..C.T...G......T....T.....T.......C...C
HT63      T.........C.CC.......C...GC.C....TA..CT....C...............T....T.....T.G.........C
HT64      T.....A...C.C........C...GC.C....T...C...C.C..C.T..........T....T...C.T.......C...C
HT65      T.....A...C.C........C...GC.C....T...C...C.C....T..........T....T...C.T.......C...C
```

The MP analysis found 17 635 shortest trees with a length of 143 steps that had a rather low consistency index and retention index (CI = 0.190, RI = 0.543), indicating the presence of substantial homoplasy in the dataset. The ratio of parsimony informative characters (49) to the number of OTUs (65) was extremely low and has additionally contributed to the MP tree being mostly unresolved. The NJ tree clearly revealed 3 major assemblages (Fig. 4.2). The first assemblage (SW) almost exclusively consisted of animals originating in the three most southwestern sample localities (A, B, and C; Fig. 4.1) and was highly supported (bootstrap value 95%). The only exception was D5, an animal from Oudtshoorn sharing HT4 with four animals from Swellendam (Table 4.1). The second assemblage (C) united animals from both central (D, E, F, G, H, and J) and northern (K, L, T, W, and Z, Fig. 4.1 and Table 4.1) sample localities. However, since approximately 60% of animals/haplotypes in this group were of central geographic origin, it will be referred to as the central group. The third assemblage (N) comprised solely animals from the northern half of the sampled area (K, L, P, S, T, U, V, W, Y, Z, Zim, and Bot; Fig. 4.1 and Table 4.1). Assemblages SW and N were characterized by a star-shaped topology with short branches, in assemblage C this trend was not as clear with some longer branches (Fig. 4.2). The three assemblages were treated as subpopulations in the further analyses. Sequence divergences ranged from 0.25% to 10.60% within subpopulations and from 1.03% to 14.04% between subpopulations (Table 4.3; for all pairwise divergences see Appendix Table A3). One animal, Z7 from Namibia, had a haplotype (HT57) that was highly divergent from all other haplotypes (6.15-13.85%) and did not consistently cluster in any of the three assemblages. Therefore, this animal was chosen as the root for the bootstrapping procedure.

Within assemblages, haplotype diversities ranged from 0.90 to 0.95, the average number of nucleotide differences was between 4.581 and 11.149, and nucleotide diversities ranged from 0.011 to 0.028, with all indices being highest in the central group (Table 4.3). These values were well within the range described for Austrian and Greek brown hares (*L. europaeus*) by Hartl *et al.* (1993) and Mamuris *et al.* (2001a, b), and for the European rabbit (*Oryctolagus cuniculus*) by Branco *et al.* (2002).

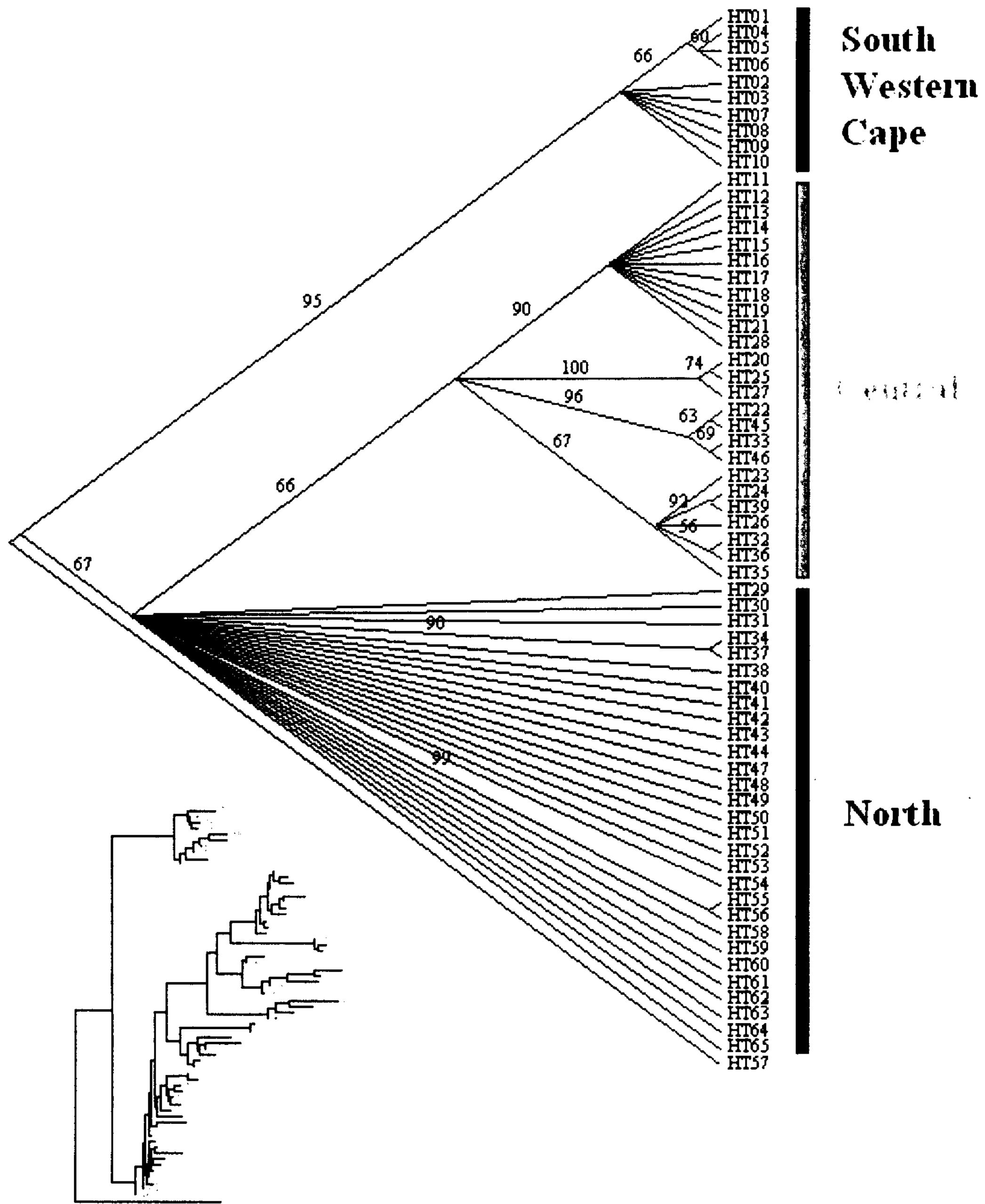

Fig. 4.2 Neighbor-joining tree (the insert shows the phylogram representation) depicting the grouping of the 65 CR-I haplotypes of *Lepus saxatilis* into three geographic lineages. The values indicate the bootstrap support for the particular internodes obtained via rooting against HT57. Haplotype designations correspond to Table 4.1, for the geographic origin of haplotypes see Table 4.1 and Fig. 4.1.

Mutation Rate and Coalescence Time. We computed the average number of nucleotide substitutions per site (*d*) and obtained a value for $d = 1.11$. Based on an average cytochrome *b* sequence divergence of 13.80% between the scrub hare and the Cape hare (see chapter 7), we estimated the interspecific divergence time by applying the standard calibration of 2-4% sequence divergence per million years (Myr) for cytochrome *b* (Martin & Palumbi 1993; Avise *et al.* 1998). From the two estimates, 6.9 Myr and 3.45 Myr, the lower value (corresponding to a divergence rate of 4% per Myr) was chosen, as it is more concordant with the fossil record. Such inferences are highly provisional as they rest upon uncertain fossil evidence. Nevertheless, they are useful standard procedures within phylogeographic studies (Avise 2000). Fossils of hares are documented for Africa from the late Pliocene/early Pleistocene (Lavocat 1978). Angermann (1973) and Chapman & Flux (1990) suggested that many leporid species originated during a rapid radiation event in the middle to late Pleistocene. Consequently, the rate of nucleotide substitutions per site per lineage per year (λ) was calculated as $\lambda = 1.11/2 \times 3\ 450\ 000 = 1.6 \times 10^{-7}$. Given a generation time in hares of two years (Marboutin & Peroux 1995), the mutation rate per nucleotide site per generation (μ) was two times $1.6 \times 10^{-7} = 3.2 \times 10^{-7}$. The mutation rate per generation per haplotype of a length of 420 bp was consequently 1.344×10^{-4}. The coalescence time in generations was determined to be 128 460 by $t = \tau/2v$ with a τ-value of 34.53 for the divergence between the scrub hare and the Cape hare (estimated in ARLEQUIN). This yielded a coalescence time to the most recent common ancestor of all southern African scrub hares of approximately 256 920 years.

Partitioning of Genetic Variation. The results of an AMOVA where the groups were categorized according to Robert's (1951) subspecies designations yielded a very low Φ_{CT} of 0.186 which was not significant ($P = 0.05$). The low contribution (18.58%) of the among group variance towards the total variance underscored the absence of differentiation among the defined groups.

In contrast, the AMOVA indicated a high degree of partitioning when the groups consisted of the three assemblages defined by the NJ analysis with a highly significant Φ_{ST} value of 0.641 (Table 4.4). The largest portion of variation 42.44% ($P < 0.0001$) of

the total variance was attributable to variation among groups (= assemblages) and Φ_{CT} was a highly significant value of 0.427. To test whether the differentiation between assemblages was mainly due to the distinctiveness of the SW assemblage, we also performed an AMOVA exclusively on animals from assemblages C and N. In this case, the Φ_{CT} value decreased to 0.299 but was still highly significant. Despite the fact that the major component of variance (45.52%) was now ascribed to within population variation, the amount of variance due to the among groups comparison was still substantial (30%). Furthermore, a highly significant Φ_{ST} value of 0.545 indicated the presence of genetic structure between the two assemblages. Pairwise comparisons of Φ_{ST} values clearly supported the distinctiveness of assemblage C from N by a highly significant ($P < 0.0001$) value of 0.332 (Table 4.5) and confirmed the very high differentiation of assemblage SW from N and C. These findings were further corroborated by the results of the exact test for population differentiation (Table 4.5). In addition, Nei's raw D_{XY} and Nei's net D_A (Table 4.6) were highly significant in all pairwise comparisons. We calculated the divergence time for the split between the three assemblages by solving the equation $t = \tau/2v$ with the different τ-values given in Table 4.5. The separation of assemblage SW from C and N occurred ± 61 235 and ± 45 685 years ago, respectively. The differentiation between assemblages C and N was more recent (± 18 080 years ago).

Table 4.3 Mitochondrial DNA CR-I diversity estimates in three phylogeographic groups of the scrub hare (SW = southwestern, C = central, N = northern); n = sample size, s = polymorphic sites, k = average number of nucleotide differences, no. of HT = number of CR-I lineages, H = haplotype diversity and π = nucleotide diversity (standard deviations are in parenthesis). Pairwise sequence divergences estimated under the HKY85 model with Γ-correction.

Assemblage	n	s	Ti/Tv	k	no. of HT	H	π	% Pairwise divergence
SW	27	19	18/0	4.581	10	0.900 (0.027)	0.011 (0.010)	0.25-2.73
C	59	62	57/3	11.149	27	0.950 (0.017)	0.028 (0.002)	0.25-10.60
N	72	62	57/7	7.974	31	0.922 (0.020)	0.020 (0.002)	0.25-8.99
total	158	83	78/9	12.251	65	0.969 (0.006)	0.031 (0.001)	0.25-14.04

Table 4.4 Hierarchical analysis of molecular variance (AMOVA) of CR-I sequences among three scrub hare subpopulations.

Hierarchy	d.f.	% Total variance	Φ Statistic		*P* value
Among groups	2	42.44	Φ_{CT}	0.424	<0.0001
Among populations	18	21.70	Φ_{SC}	0.377	<0.0001
Within populations	138	35.86	Φ_{ST}	0.641	<0.0001

Table 4.5 Mitochondrial DNA CR-I pairwise Φ_{ST} values for comparisons of scrub hare subpopulations (SW = southwestern, C = central, N = northern) and significance levels (lower diagonal), *** indicates $P < 0.0001$; and exact test for population differentiation based on haplotype frequencies at significance level of $P = 0.05$ (+ indicates significant differentiation) and interpopulational divergence times, τ (upper diagonal).

	SW	C	N
SW		+/8.23	+/6.14
C	0.576/***		+/2.43
N	0.566/***	0.332/***	

Table 4.6 Average pairwise differences (Nei & Li 1979) among scrub hare subpopulations (SW = southwestern, C = central, N = northern), Nei's raw D_{XY} (upper diagonal), Nei's net D_A (lower diagonal), and intrapopulational Nei's D_X as bold diagonal elements (all pairwise comparisons at a significance level of $P < 0.0001$); results of neutrality tests, all not significant.

	Pairwise Differences			Neutrality Tests		
	SW	C	N	Tajima's D	Fu & Li's D*	Fu & Li's F*
SW	**4.655**	23.063	16.949	-0.06688	-0.04926	-0.06415
C	14.237	**12.997**	16.244	-0.01294	0.60593	0.44625
N	10.183	5.307	**8.877**	-1.10138	-1.74867	-1.79118
Total	-	-	-	-0.16477	-0.56622	-0.46631

Migration and Population Sizes. Estimates of migration rates among the three subpopulations indicated the absence of gene flow between SW and N. The exchange of migrants between SW and C was small in both directions (0.2375 from SW to C and 0.2223 from C to SW). The C group received 0.918 female migrants per generation from the N group which in turn received 1.8501 from C. Neutrality statistics were consistently non-significant (Table 4.6), confirming the assumption of selective neutrality of the investigated CR-I sequences. Furthermore, the θ-estimators obtained in MIGRATE were

very close to the values of nucleotide diversity (π, Table 4.3) and thus suggestive that the populations were in genetic/demographic equilibrium. Based on the estimated mutation rate for the CR-I sequences of the scrub hare and the θ-values from MIGRATE, we calculated the current effective female population sizes to be 25 359 for SW, 94 515 for C, and 80 796 for N.

Mismatch Distributions.

The frequency distributions of pairwise nucleotide differences were investigated respectively for four different samples, for the entire scrub hare population (Fig. 4.3), as well as for the three assemblages separately (Figs. 4.4-4.6). All observed mismatch distributions were unimodal and corresponded to the expected distributions generated under the sudden expansion model. Most goodness-of-fit statistics (sum of squared deviation and Harpending's raggedness index) were not significant and thus the null hypothesis could not be rejected. Only Harpending's raggedness index for the C assemblage was significant. However, this index still had a rather small value (0.021), indicative of a unimodal distribution (Harpending 1994). The sum of squared deviation for this group did not support a departure from the null hypothesis of population expansion (Fig. 4.5). The θ-values (which are directly proportional to the effective maternal populations sizes) for all cases confirmed the scenario of population expansions, the initial θ-values were always much smaller than the final θ-values (Table 4.7). The mean number of pairwise differences was 16 for the total population, five for the SW group, 15 for the C group, and seven for the N group. Given a mutation rate per haplotype per year of 0.672×10^{-4} (see above), and dividing the average number of mutations per lineage by the mutation rate, approximate estimates for the timing of the expansions were calculated as 119 048 years ago for the entire sample, 37 202 years ago for the SW assemblage, 111 607 years ago for the C assemblage, and 52 083 years ago for the N assemblage. As a control, we estimated the time to population expansion by solving the equation $\tau = 2\nu t$ for t and applying the mismatch analyses derived τ-values from ARLEQUIN (Table 4.7). The outcomes were similar indicating 130 505, 42 559, 125

595, and 45 684 years back to the expansion event for the entire population, the SW group, the C group, and the N group, respectively.

Table 4.7 Values of θ_0, θ_1, and τ for three scrub hare subpopulations (SW = southwestern, C = central, N = northern) and for the total population estimated via three different approaches. Watterson's procedure describes θ per gene, the MHMC method describes θ per site (see text for details).

	Watterson/DNASP		MHMC/FLUCTUATE		N-L L-S/ARLEQUIN		
	θ_0	θ_1	θ_0	θ_1	θ_0	θ_1	τ
SW	1.593	4.670	0.012	0.021	0.004	15.691	5.72
C	5.458	12.755	0.032	0.101	0.000	41.768	16.88
N	4.620	11.192	0.030	0.078	5.492	15.996	6.14
Total	4.991	12.936	0.035	0.225	0.006	47.805	17.54

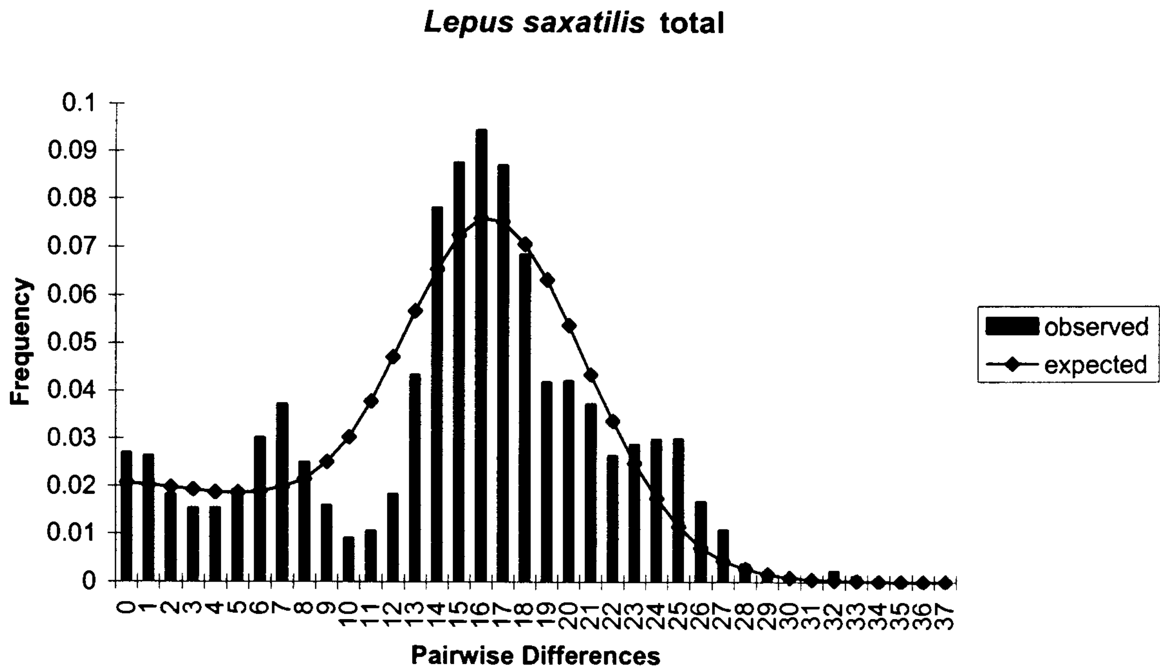

Fig. 4.3 Mismatch frequency distribution of the pairwise nucleotide differences in the total population of scrub hares, sum of squared deviation = 0.005 (P = 0.315) and Harpending's raggedness index = 0.004 (P = 0.495).

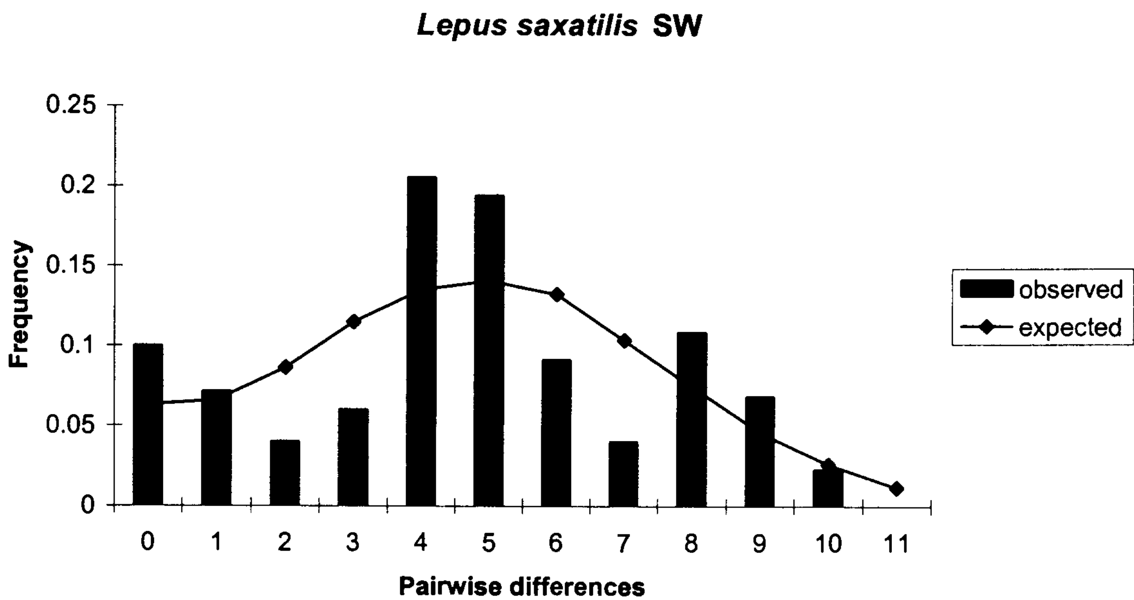

Fig. 4.4 Mismatch frequency distribution of the pairwise nucleotide differences in the SW group, sum of squared deviation = 0.021 (P = 0.148) and Harpending's raggedness index = 0.045 (P = 0.208).

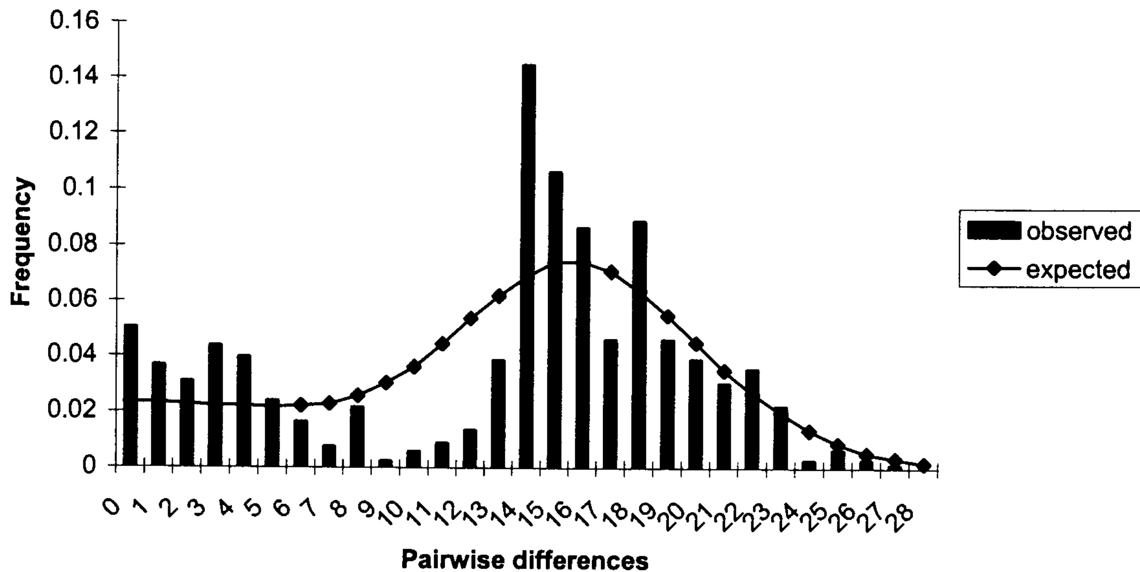

Fig 4.5 Mismatch frequency distribution of the pairwise nucleotide differences in the C group, sum of squared deviation = 0.015 (P = 0.065) and Harpending's raggedness index = 0.021 (P = 0.019).

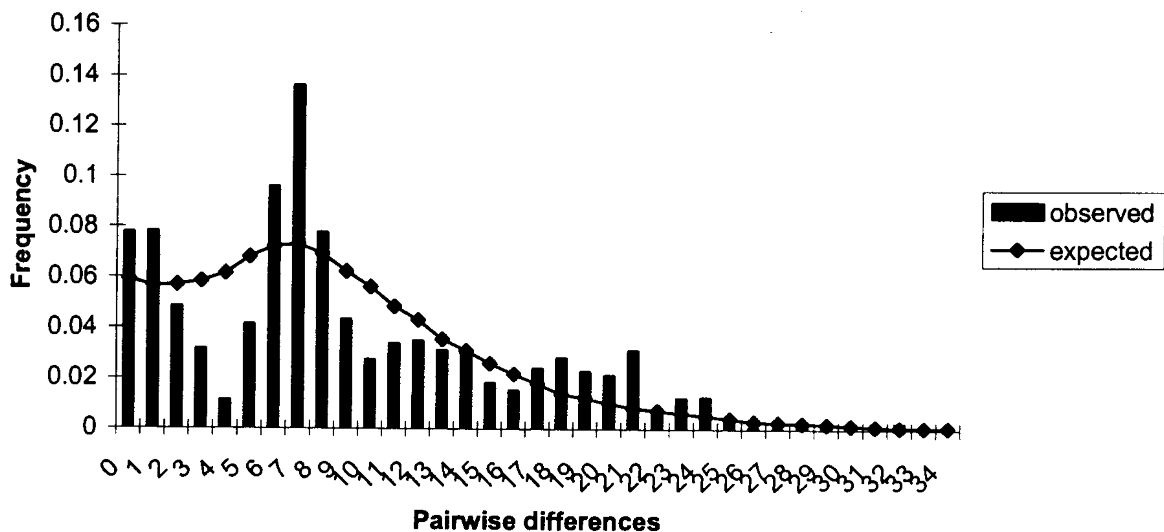

Fig. 4.6 Mismatch frequency distribution of the pairwise nucleotide differences in the N group, sum of squared deviation = 0.013 (P = 0.446) and Harpending's raggedness index = 0.013 (P = 0.578).

4.5 Discussion

Maternal lineages

The variation of mtDNA in the scrub hare identified three separate maternal lineages that roughly coincide with three different geographical areas (Fig. 4.2). There is a marked genetic discontinuity separating the SW group from the other two groups with Nei's net $D_A > 10$ (Table 4.6) and sequence divergences over 14%. This is indicative of a long-term historical separation and could be due to a significant zoogeographic barrier to dispersal (Avise *et al.* 1987; Avise 2000). There is no readily apparent extrinsic barrier separating SW from C or N. However, Vrba (1985) emphasized that gross physical as well as subtle ecological barriers are highly dynamic and appear and disappear over historical time frames. Avise *et al.* (1987) suggested that intraspecific phylogeographic discontinuities in mtDNA are likely to align with zoogeographical provinces. The distribution of SW partly overlaps with the Fynbos Province as described by Werger (1978) and fits even more closely to the zoochorological Cape zone as defined by Carcasson (1964). Alternatively, isolation could be maintained by intrinsic barriers such as socio-ecological behavior as speculated by Mamuris *et al.* (2001). The divergence times of 61 235 and 45 685 years ago from C and N, respectively, substantiate the hypothesis of a long-term isolation of SW. The more recent split between C and N some 18 080 years ago is concordant with the relatively lower D_A value of 5.307 (Table 4.6).

Environmental changes that might lead to vicariance can often be connected to climatic changes (Vrba 1985). Tyson & Preston-Whyte (2000) recorded multiple major climatic shifts in Africa towards cool and dry conditions between 70 000 and 20 000 years ago. During those colder periods the forests contracted and historical scrub hare populations are likely to have expanded their ranges into the newly available open savanna habitats. Yet, the whole period was characterized by rapid climatic oscillations (Kingdon 1990). Therefore, the newly acquired habitats were periodically fragmented again during the more humid and mesic intervals which could have easily led to vicariance at the calculated times.

The relatively high sequence divergences (up to 11.90%) between the more closely related assemblages C and N are partly resulting from the very high intrapopulational variation present in C (up to 10.6% sequence divergence; Table 4.3; and a value of Nei's D_X of nearly 13; Table 4.6). Within the groupings N and SW, the phylogeographic structure was shallow (Fig. 4.2) suggesting recent range expansions emanating from different refugial populations. Newly arisen mutations that have not yet had enough time to spread through the entire distribution range then account for the high incidence of closely related haplotypes specific to certain localities and the starlike phylogeny obtained herein (Fig. 4.2). Furthermore, haplotypes drawn from the populations have not yet been sorted by stochastic lineage extinction, and reciprocal monophyly has not yet been reached (Avise *et al.* 1984, 1990; Avise & Wollenberg 1997).

By interpreting the molecular data resulting from this study, it is possible to speculate on the approximate locations of the refugial populations. Generally, ancestral haplotypes within assemblages are likely to be more common than others and geographically more widespread (Watterson & Guess 1977; Avise *et al.* 1987). The most frequent haplotype with the widest geographical distribution within the SW assemblage was HT4, which occurred five times over a distance of 202 km. Swellendam (B; Fig. 4.2) would thus be a likely area for the SW refugium. Within the C group, HT11 was found in 11 individuals over a distance of 250 km and would therefore qualify as the most likely candidate for an ancestral haplotype. This rendered the area around Oudtshoorn and Loxton (D and E; Fig. 4.2) as a possible refugium for C. In N two haplotypes (HT31 and HT40) occurred with the same high frequency of 14 individuals each. Since HT31 covered a much larger area (over 700 km in diameter), it was more likely to be the ancestral haplotype. In any case, both haplotypes were prevalent in the area around Pretoria–Rustenburg (T and U; Fig. 4.2), which therefore is proposed as the refugium for this assemblage.

Coalescence time

The calculated mutation rate per nucleotide site per generation, $\mu = 3.2 \times 10^{-7}$, corresponded to a sequence divergence of 32% per Myr (using a generation time of two years). Vigilant *et al.* (1991) proposed a CR sequence divergence of 17.3% per Myr for

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humans; Pierpaoli *et al.* (1999) suggested 12.4% per Myr for European hares. The fact that the substitution rate calculated in this study was higher than the rate described for humans may be due to the higher metabolic rate of hares associated with their smaller body size (Martin & Palumbi 1993). On the other hand, Ward *et al.* (1991) also found a mutation rate for human CR sequences of 33% per Myr.

The estimated coalescence time for the southern African scrub hare population based on the mutation rate of 3.2×10^{-7} was with approximately 256 920 years in the range of estimates for other vertebrates species (Rooney *et al.* 2001). If we used the estimates of slower control region substitution rates (see above), we obtained coalescence time estimates for the whole scrub hare population of 475 620 and 664 038 years, respectively. At first glance, these values seem closer to a probable Pliocene/Pleistocene origin for the species. The date retrieved by applying the mutation rate suggested by our data set, however, also coincides with the expected time frame and is used in preference, since it was specifically determined from the sequence data contained herein. It must further be considered that the time scale of the coalescent is dependent on the effective population size (Wakeley 2000). Therefore, the TMRCA as inferred from mtDNA might be an underestimation due to the absence of recombination and the reduced population size of this marker (Grant & Leslie 1993). Furthermore, a speciation event may seem more recent than it actually occurred if the migration rate among populations within the species is low, as is the case between SW and the other two populations in the scrub hare (see below).

However, gene trees are not necessarily congruent with the species trees (Brower *et al.* 1996; Maddison 1997; Walker & Avise 1998; Nichols 2001), and gene divergences do not coincide with the actual species divergences. Thus the genealogy of one gene may well be inconsistent with the phylogeny of the species under investigation (Avise 1989; Wakeley 2000). Future research should therefore include the assessment of genealogies of multiple independent and nuclear loci (Edwards & Beerli 2000).

calculations in FLUCTUATE allow for historical fluctuations in populations sizes and are therefore used in arguments presented below. It is suggested that after the expansion, the effective maternal population size for the entire *L. saxatilis* population numbered approximately 351 563 individuals. The southwestern population experienced a size expansion some 37 202-42 559 years ago and grew from initially 18 750 animals to 32 813. The expansion event in the central population occurred 111 607–125 593 years ago and caused a population increase from 50 000 to 157 813 animals. For the northern population, the expansion was dated back to 45 684–52 083 years ago with an increase in size from 46 875 to 121 875 female hares. The expansion events all date back to the late Pleistocene and fall into the period from 125 000-16 000 years before present. This coincides with a period of rapid and pronounced warmings followed by slow temperature declines (Tyson & Preston-Whyte 2000). Sudden ecological changes in habitats due to rapid temperature shifts might have precipitated the sudden population size expansions in the scrub hare.

A final line of evidence which can be invoked in ascertaining patterns and processes of past expansions includes neutrality tests. These are not only sensitive to non-neutral evolution, but also to population history (Tajima 1989b; Fu & Li 1993), and negative signs of these statistics are compatible with events of population expansion and/or bottlenecks (Tajima 1989b; Simonsen *et al.* 1995). In this light, the negative values for Tajima's D, Fu & Li's D* and F* (Table 4.6) are in agreement with the results of the mismatch distribution analyses.

Three different bodies of evidence suggest that the three *L. saxatilis* populations detected in this study are the result of relatively recent population expansions. The star like phylogeny depicted in the NJ tree (Fig. 4.2) further supports this. Admittedly however, the genetic signal of a size expansion was somewhat ambiguous in assemblage C. On one hand, Harpending's raggedness index was significant (Fig. 4.5) indicating a rejection of the null hypothesis of a sudden population expansion. On the other hand, Tajima's D had a negative value (Table 4.6) signifying an expansion event. This ambiguity was probably due to the comparatively long time lapse since the expansion (111 607–125 593 years ago, $\tau = 16.88$; Table 4.7) in combination with the high intrapopulational diversity (up to 10.6% sequence divergence; Table 4.3; and Nei's D_X =

Migration rates

According to the standard interpretation of migration rates, values below one migrant per generation are considered to be insufficient to overcome genetic divergence among populations caused by drift (Griswold & Baker 2002). Thus, the estimates of gene flow resulting from the MIGRATE analyses presented here suggested that the SW group is genetically isolated from the other two groups. The finding of a shared haplotype (HT4) between SW and C (Table 4.1) would consequently have to be interpreted as the signature of shared ancestry between these two populations. The only significant ongoing gene flow connection among the three populations of scrub hares exists from C to N (1.8501 female migrants per generation). Bearing this in mind, the animal K2 collected at the northern locality K (Willem Pretorius Game Reserve; Table 4.1) was likely to be an immigrant from the central areas, since it shared haplotype HT20 with the specimens G1 and G5 from the central locality G (Tussen-die-Rivieren Game Reserve), and this haplotype clustered within the central assemblage (Fig 2). In addition, a number of animals collected in the northern localities Vryheid, Ermelo, and Hoedspruit (L1, L5, L6, P4, Bot, T1, W1, and W3; Table 4.1) genetically belonged to the central cluster (HT33, HT35, HT36, HT39, HT45, and HT 46; Table 4.1 and Fig. 4.2) and could therefore also be understood as immigrants into the North. Since we did not have more samples from Botswana and the central northern parts of South Africa, it was not possible to draw a clear-cut geographical border between the C and N assemblages. It is quite possible that the range of the central assemblage actually extends all the way up into Botswana.

Overall, the amount of female mediated gene flow among the populations is surprisingly low for a species described as highly vagile (Angermann *et al.* 1990; Schneider 1990). In addition, 53 of the 65 CR-I haplotypes (approximately 82%) were private to single sampling localities. This may indicate that (at least female) scrub hares prefer to remain in their home ranges and only move over long distances in case of extreme environmental pressure (Hulbert *et al.* 1996). This would be in agreement with Mamuris *et al.* (2001a,b) who reported the lack of migration in Greek brown hares (*L. europaeus*). Alternatively, the low migration rates detected in this study may be due to the marker used which does not register male-biased dispersal. Avise (1995)

mentioned the high frequency of such gender-biased gene flow in mammals, and Prugnolle & deMeeus (2002) stated that in mammals dispersal is usually male-biased. More specifically, Reitz & Leonard (1994) found that females of the European hare (*L. europaeus*) are more sedentary than males. In general, therefore, estimates of the number of migrants between the populations have to be interpreted cautiously all the more so since non-zero values can also be produced by retention of ancestral polymorphism (Templeton & Georgiadis 1996; Griswold & Baker 2002).

Population size expansions and colonization scenario

With an average haplotype diversity of 0.969, the South African scrub hare exhibits a high degree of genetic polymorphism comparable to that detected in European hares (Mamuris *et al.* 2001a,b). Haplotype diversities (Table 4.3) are consistently high for the total sample (0.969), as well as for the three assemblages (0.9–0.95). The nucleotide diversities for the species in its entirety, for C, and for N are rather low (3.1%, 2.8%, and 2%, respectively) and very low for SW (1.1%; Table 4.3). This is indicative of shallow divergences and, in combination with high haplotype diversities, bears testimony to rapid population growth from bottlenecked ancestral populations (Avise 2000).

Signatures of sudden population size expansions were also reflected in the results of the mismatch distribution analyses (Figs. 4.3-4.6). The estimates of the historical effective female populations sizes derived from three different calculation procedures (θ; Table 4.7) differed in exact values but nevertheless consistently revealed strong increases in the population sizes. For the entire sample, a size expansion was inferred to have happened 119 048-130 505 years ago. The initial effective female population size was around 54 688 as calculated from the genealogical relationships in FLUCTUATE, or 18 568 as calculated from the number of segregating sites in DNASP. The markedly different estimates underscore the importance of interpreting these data as relative indicators and not as precise numbers. Moreover, the values derived from DNASP are probably underestimates, since the sequence data exhibited a high degree of homoplasy and the assessment of the number of segregating sites in DNASP does not account for multiple hits. The trends were identical in all different estimation procedures (Table 4.7). The

12.997; Table 4.6) of the central population. Based on these facts, it can be speculated that C represents the ancestral population of scrub hares for South Africa. During the numerous climatic changes in the Pleistocene, the habitat in the southwest and the north may have become more suitable at certain stages and small founder populations may have split off and spread out to occupy those newly available areas. Subsequently, they were isolated from the central source population in the course of renewed climatic changes. Those founder populations for SW and N were most probably smaller than the large central source population and consequently may have lost their already lower genetic variation more easily during consecutive range contractions. This may explain the retention of the ancestral polymorphism in C, but not in SW or N.

Subspecies boundaries

Wiley (1981) and Frost & Hillis (1990) suggested that the taxonomy of a group should be consistent with its evolutionary history. None of the subspecies designations based on coloration were supported as evolutionarily meaningful units by the mtDNA data presented here. The molecular based phylogeny of the species instead revealed the existence of three geographically distinct assemblages within *L. saxatilis* each of which reflected different demographic histories. Animals belonging to the same subspecies category clustered in different molecular phylogenetic assemblages (*L. s. saxatilis* had representatives in SW & C, *L. s. orangiensis* occured in C & N). On the other hand, assemblages C and N were made up of animals from several subspecies that did not group separately according to their designations within the assemblages. The phylogeographic unit SW coincided somewhat with the morphologically defined, colloquially named "Ribbokhaas" (a very large animal with very long ears, attributable to the subspecies *L. s. saxatilis* and *L. s. megalotis*). However, the genetically defined taxon appears to have a much more restricted geographic distribution. The subspecies categories for the scrub hare, therefore, seem to represent color pattern classes that are more related to ecological habitat, than to evolutionary processes.

The uniqueness of the (SW) assemblage merits further discussion. The mtDNA data suggest that it is an independent maternal lineage without any recent gene flow connections to populations C and N. It has been isolated for more than 45 000 years and may warrant distinct status under various species concepts. Mayr (1963) and Dobzhanzky (1970) originally defined a species under the Biological Species Concept by the absence of gene flow while the Phylogenetic Species Concept (Cracraft 1989; Nixon & Wheeler 1990; Davis & Kevin 1992; Davis 1996) considers a species to be a distinct lineage with consistently diagnosable traits, which is basically determined by the presence or absence of gene flow. Additionally, Templeton's Cohesion Species Concept (Templeton 1989, 2001) posits that populations of a species comprise a single evolutionary lineage that is defined as a field of gene recombination (gene flow). As the SW group lacks any gene flow connections to the other two lineages, it could conceivably be addressed as a biological species, as a phylogenetic species, or even a cohesion species. However, the biological species concept does not seem adequate for hares (many well defined species hybridize with fertile offspring; e.g. Thulin *et al.* 1997). Moreover, it is not possible to define a cohesion species exclusively based on molecular data, since the criterion of ecological exchangeability cannot be addressed. In terms of the phylogenetic species concept, we define the SW group of scrub hares as a separate species, in as much as it forms an irreducible and diagnosably distinct cluster of organisms with an independent genealogy (cf. Cracraft 1983).

Chapter 5

Phylogeography of the South African Scrub Hare (*Lepus saxatilis)* inferred from a combined analysis of mitochondrial DNA and microsatellites

5.1 Abstract

The scrub hare, *Lepus saxatilis*, of southern Africa has recently been regarded as comprising three distinct maternal lineages based on neighbor-joining analyses of mitochondrial DNA control region (mtDNA CR) sequence data. We re-analyzed these sequence data with nested clade analysis and additionally conducted analyses with a suite of six polymorphic microsatellite markers on the same sample of 159 hares. The species subdivision into three phylogroups was confirmed. The depth of the mitochondrial gene tree and the significance in allele frequency differences at the microsatellite loci indicated that one phylogroup, SW, represents an evolutionarily significant unit (ESU) and the other two populations, C and N, delimit management units (MUs) within a second ESU. Discordance in the estimated number of migrants from the two different marker sets suggested male-biased dispersal. The data suggested that the evolutionary history of the species was influenced mainly by past fragmentation events and contiguous range expansions. The geographic location of one of the two identified phylogeographic discontinuities coincided with a break described for other southern African small mammals.

5.2 Introduction

The genetic architecture of an extant species reflects the effects of historical biogeographic factors, as well as contemporary ecologies and behaviors of the organism under investigation (Avise *et al.* 1987; Avise 2000). Phylogeography is concerned with the influence of historical factors such as dispersal events and periods of vicariance on the geographic distribution of gene lineages (Avise 2000). Many species display a significant phylogeographic structure with members of different geographic populations occupying recognizable genealogical branches of the intraspecific pedigree. A limited ("shallow") phylogeographic structure in a species is usually attributed to a life history characterized by high dispersal capabilities and attendant immunity to historical (biogeographic) barriers to gene flow.

Hares are generally thought to be highly vagile with single animals easily moving distances of up to 20 km per night and over longer time periods individuals have been reported to move distances of up to 400 km (Schneider 1990). Whole populations can shift hundreds of kilometers in response to environmental changes or in search of better feeding grounds, and there are numerous reports of "hare migrations" where large populations travel together (Angermann *et al.* 1990; Schneider 1990). Given this high degree of mobility, one would expect to find a panmictic population structure in many of these species. Contrasting phylogeographic patterns have been observed in *Lepus* species. Suchentrunk *et al.* (1999) and Suchentrunk & Alves (2001) noted no phylogeographic partitioning in European brown hares (*L. europaeus*), mountain hares (*L. timidus*), and Iberian hares (*L. granatensis*). These findings are, however, in conflict with those of Perez-Suarez *et al.* (1994); Thulin *et al.* (1997); and Mamuris *et al.* (2001a, b) who all reported populations of the European brown hare (*L. europeaus*) to be highly differentiated. Kryger *et al.* (submitted; chapter 4) investigated the geographic partitioning of genetic variation among southern African scrub hares (*L. saxatilis*) by sequencing 420 bp of the hypervariable 5' end of the mitochondrial control region (CR-I). A neighbor-joining [NJ] analysis of the highly polymorphic sequence data identified three major maternal lineages of scrub hares that were more or less restricted to different geographic regions in southern Africa: a southwestern assemblage, a central and a

northern assemblage (Fig. 5.1). The southwestern group seemed highly distinct and historically isolated from the other two scrub hare populations. This was interpreted to be the result of a vicariance event in the Holocene. While AMOVA analyses confirmed the validity of the geographic substructuring of the mtDNA CR-I variation, two of the three geographical groupings were not supported by high bootstrap values in the NJ and MP trees. This was thought to result from the large amount of homoplasy in the data. The question remained whether all three lineages are discrete genealogical units with independent demographic and evolutionary histories.

Smouse (1998) argued that most intraspecific molecular data sets are highly homoplasic and therefore phylogenetically ambiguous. Since molecular markers for phylogeographic studies are selected precisely because of their high mutation rate (and informativeness), these data sets are often characterized by multiple evolutionary substitutions that are identical in state, but not by descent (Excoffier & Smouse 1994; Bandelt *et al.* 1995, 1999). Smouse (1998) therefore argued that imposing phylogenetic trees upon population data that are not credibly tree-like is inappropriate. Clement *et al.* (2000) also critisized the use of traditional methods of phylogenetic reconstruction (such as NJ, MP, and maximum likelihood [ML]) for intraspecific relationships since these methods are based on assumptions that are invalid at the population level. In contrast with the macroevolutionary context, at the microevolutionary level ancestral haplotypes might still be present in the population; in fact, coalescent theory predicts that these will be the most frequent sequences in a population sample (Watterson & Guess 1977; Donelly & Tavare 1986; Crandall & Templeton 1993). Furthermore, intraspecific genealogies are often characterized by multifurcations and reticulations, features not catered for in traditional phylogenetic trees (Posada & Crandall 2001). Following the method of cladogram estimation introduced by Templeton *et al.* (1992), Clement *et al.* (2000) developed a software package (TCS) that estimates gene genealogies specifically at population level. TCS outputs an unrooted cladogram represented as a network consisting of 95% parsimoniously plausible branches connecting haplotypes. Templeton *et al.* (1987) and Templeton & Sing (1993) introduced nesting rules for determining a nested structure of the clades from a haplotype network and subsequently, Templeton *et al.* (1995) described a method of analyzing these nested clades such that historical and

contemporary processes can be separated from each other. This nested clade analysis (NCA) can detect possible deficiencies in the sampling regime and can test for the underlying evolutionary dynamics (past fragmentation events, population range expansions, or recurrent genetic drift coupled with gene flow) responsible for any observed spatial patterns within the genetic variation. In the present study, we analyzed the mtDNA sequence data of Kryger *et al.* (submitted; chapter 4) with these innovative methods developed specifically for the analyses of intraspecific processes. Our aim was to retrieve more detailed and precise information on the evolutionary processes shaping the genetic variation in *L. saxatilis* in southern Africa.

Several authors have emphasized that major splits in gene trees do not necessarily coincide with deep separations in population or species trees (Avise 1995; Brower *et al.* 1996; Maddison 1997; Walker & Avise 1998; Nichols 2001). The phylogenetic partitions in gene trees are usually deeper than the shallow historical partitions in organismal phylogenies (Avise 1998) and the coalescence time (time to the most recent common ancestor, TMRCA) of two DNA sequences is typically longer than the coalescence time of two species (Nichols 2001). Avise (1998) recommended looking for the presence or absence of concordance in significant genealogical partitions across several unlinked loci within a species, and then drawing conclusions from combined sets of evidence. Avise & Ball (1990) and Walker & Avise (1998) argued that evidence of genealogical concordance must be sought to support inferences that major phylogenetic branches within a gene tree actually depict major branches in an organismal phylogeny. Likewise, Grant & Leslie (1993) urged that population genetic studies should include both nuclear and mitochondrial markers, since analyses of mtDNA alone might fail to detect distinct populations or cryptic species. These authors further explained that especially in semi-arid environments such as South Africa (where species are generally confronted with many cycles of extinctions and recolonizations), the loss of genetic variability due to genetic drift is generally significantly higher in mtDNA than in nuclear DNA as the effective population size for organellar genes is only one quarter that of nuclear genes (Grant 1993). Microsatellites are nuclear markers that are widely used for assessing levels of genetic variation in population studies (Bruford & Wayne 1993; Paetkau & Strobeck 1994; Paetkau *et al.* 1995, 1997). In order to characterize the genetic status of

South African scrub hare populations on a more comprehensive basis, we therefore undertook a microsatellite analysis as presented here.

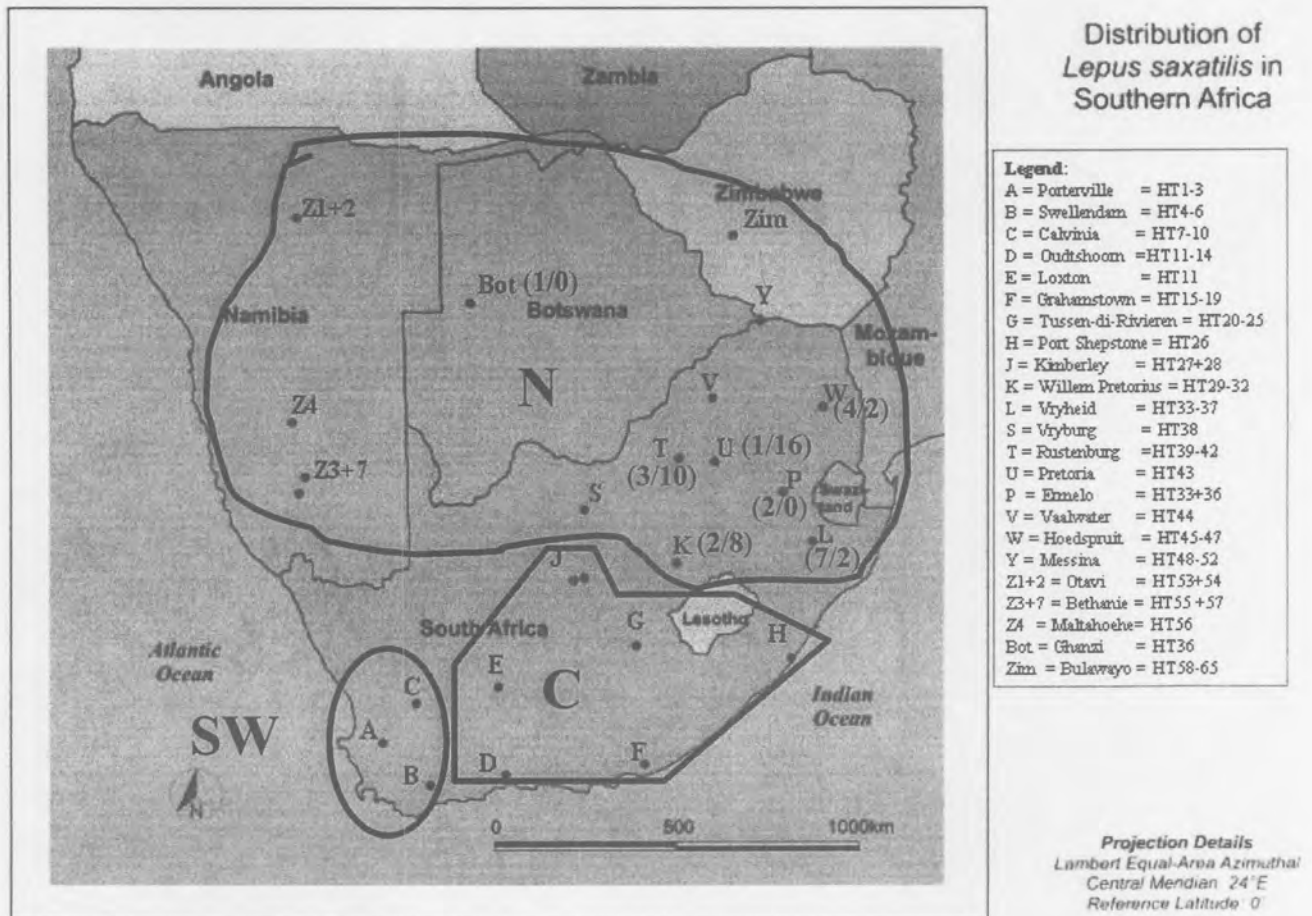


Fig. 5.1 Sampling locations of *Lepus saxatilis* and CR-I haplotypes found at specific locations. The encircled areas correspond to the three geographic assemblages (SW, C, and N) identified in Kryger *et al.* (submitted; chapter 4). The number of animals with haplotypes clustering in the central/or northern group in the neighbor-joining tree based on mitochondrial CR-I sequences are shown in brackets.

The IUCN (International Union for Conservation of Nature and Natural Resources) lagomorph specialist group emphasized the importance of lagomorphs as medium-sized herbivores that sustain a multitude of predators in many ecosystems (Chapman & Flux 1990). As a prerequisite for adequate conservation decisions within the genus *Lepus*, Chapman & Flux (1990) underscored the need to clarify the still confused systematics and taxonomy at species and subspecies level. They called for molecular studies aiming to identify genetically distinct forms that should then be considered conservation priorities. Especially in cases where the existing taxonomy does not amply reflect the underlying genetic diversity, the concepts of evolutionarily significant units (ESUs) and management units (MUs) have been praised as providing an objective framework for prioritizing units for protection (Fraser & Bernatchez 2001). Different authors have proposed different criteria for the definition of ESUs and MUs (Ryder 1986; Waples 1991; Dizon *et al.* 1992; and Vogler & DeSalle 1994). Among the most stringent, and certainly objectively testable definitions are those developed by Moritz (1994a, b). This author suggested historically isolated sets of populations form ESUs, since they possess distinct evolutionary potential. He provided two criteria for assessing whether a population warrants ESU status: (i) reciprocal monophyly for mtDNA alleles and (ii) significant divergence of allele frequencies at nuclear loci. Moritz (1994a, b, 1999) defined MUs as currently demographically independent populations that should be recognized on the grounds of diverging allele frequencies at mtDNA and/or nuclear loci. For the long-term maintenance of evolutionary processes within a species, Moritz (1995, 1999) considered it crucial that the major elements of intraspecific diversity as represented in ESUs should be preserved. For the short-term management of a species, Moritz (1995, 1999) recommended that MUs be monitored separately but allowing translocations among them if necessary. Since it has been shown that the subspecies taxonomy of *L. saxatilis* does not reflect the distribution of intraspecific genetic diversity within the species (Kryger *et al.* submitted; chapter 4), the identification of ESUs and/or MUs would represent a valuable tool for meaningful conservation and management decisions for the scrub hare.

The aims of this study were: (i) to interpret the geographical distribution of mtDNA lineages quantitatively in a coalescent-based framework and identify the evolutionary

processes responsible for the observed intraspecific mtDNA substructuring in the scrub hare, (ii) to quantify and compare the levels of nuclear diversity for the total southern African population of scrub hares and for each of the three subpopulations; (iii) to estimate the amount of gene flow among the three subpopulations from biparentally inherited markers, (iv) to compare the phylogeographic pattern of *L. saxatilis* to that of other lagomorphs and South African small mammals, and (v) to relate the nuclear genetic differentiation to the results from the mtDNA sequencing data and identify ESUs and MUs within the species.

5.3 Materials and methods

Data collection mtDNA

The mitochondrial control region (CR-I) sequence data generated by Kryger *et al.* (submitted; chapter 4) were used.

Data collection microsatellites

Six microsatellite loci (Lsa1, Lsa2, Lsa3, Lsa4, Lsa6, and Lsa8) isolated and characterized for the scrub hare (*L. saxatilis*) by Kryger *et al.* (in press; chapter 2) were amplified and scored. Polymerase chain reactions (PCR) for all 159 scrub hares and all six loci were performed as described by Kryger *et al.* (in press; chapter 2). Products were run on an ABI 377 automated sequencer and scored against GENESCAN-500 Tamra (PE, Applied Biosystems) as the internal size standard using the GENESCAN and GENOTYPER software (PE, Applied Biosystems).

Data analysis mtDNA

We estimated a 95 % parsimony cladogram for the mtDNA CR-I data in TCS (Clement *et al.* 2000) treating gaps as a fifth character state. Loops (=reticulations) in the network, which resulted from the homoplasy in the data, were broken in accordance with the predictions derived from coalescent theory by Donelly & Tavare (1986), Crandall & Templeton (1993), and Posada & Crandall (2001): (i) Common haplotypes are more likely to be found at interior nodes of a cladogram, and rare haplotypes at the tips; (ii) haplotypes represented by only one individual (singletons) are more likely connected to haplotypes from the same population than to haplotypes from different populations.

Based on this cladogram, the nested clade structure for our data set was determined following the nesting rules described in Templeton *et al.* (1987) and Templeton & Sing (1993). Such a nested design treats haplotypes as "0-step clades", groups of haplotypes separated by a single mutation as "1-step clades", groups of "1-step clades" separated by a single mutational step as a "2-step clade" and so on. As the data set of this study was characterized by the presence of many missing haplotypes, the nesting was done by collapsing long branches connecting extant/sampled haplotypes and ignoring the missing haplotypes. Thus the number of nesting levels could be kept in a reasonable dimension. Since large numbers of mutational steps between sampled haplotypes mainly occurred at the tips of the cladogram, the evolutionary signal was not distorted (see Durand *et al.* 1999).

A nested clade analysis applying the methods described in Templeton *et al.* (1995) was performed using the program GEODIS (Posada *et al.* 2000). This program treats the sample locations as categorical variables and performs exact permutational contingency tests against the null hypothesis of no association between genetic variation and geographical distribution. Furthermore, it uses the geographical coordinates of each population to calculate two statistics, the clade distance (D_c) and the nested clade distance (D_n). The clade distance, D_c (X), measures the geographical spread of clade X and equals the average distance of individuals in clade X from the geographical center of this clade. The nested clade distance, D_n (X), measures how clade X is geographically distributed relative to sister clades within the same higher-level nesting category, and equals the

average distance of individuals from clade X from the geographical center of the higher-level nesting clade. Additionally, the difference between the average interior distance and the average tip distance within each nesting level (I-T D_c and I-T D_n) was determined. Under the expectations derived from neutral coalescent theory (Crandall & Templeton 1993), this contrast of interior clade *versus* tip clade corresponds to old *versus* young and common *versus* rare. We estimated whether any of the D_c or D_n values were significantly small or large at the 5% level using the Monte Carlo procedure described by Roff & Bentzen (1989) with 10 000 resamples. The results of the NCA were interpreted according to Templeton *et al.* (1995), Crandall & Templeton (1996), and the new NCA key inference (2001), which was downloaded from the NCA webpage (http://zoology.byu.edu/crandall_lab/geodis.htm).

Data analysis microsatellites

It is thought that the evolutionary forces of drift and mutation will lead to divergences of microsatellite allele frequencies among different populations (Murray 1996). Migration, on the other hand, will homogenize allele frequencies. In the presence of natural selection, signatures of these processes will be blurred. Microsatellites are supposedly neutral markers; they may, however, be linked to selected loci (Murray 1996). Therefore, the first step in analyzing the microsatellite data was to test for selective neutrality of each of the loci. This was done by comparing the observed frequencies of genotypes to those expected under Hardy-Weinberg equilibrium (HWE). The larger the number of loci containing an independent history the greater the chance of detecting population substructure (Murray 1996). If several loci are genetically linked, the results obtained from a study may be biased towards the history of that specific linkage group (Murray 1996). For this reason, linkage analysis was performed to test for the independent assortment of genotypes at different loci. POPGENE Version 1.31 (Yeh *et al.* 1997) was used to compute expected genotypic frequencies under random mating using the algorithm by Levene (1949) and to perform likelihood ratio (G^2) tests for HWE at each locus. We used Fisher's exact test as implemented in GENEPOP Version 3.2a (Raymond & Rousset 1995a) to check for linkage disequilibrium between loci. The exact *P*-values of

the null hypothesis of independent assortment of genotypes at different loci within populations were estimated using a Markov chain method (with 10 000 dememorizations, 1000 batches, and 10 000 iterations per batch). Furthermore, a global test for genotypic disequilibrium across all populations was performed for all pairs of loci. For *a posteriori* significance testing of linkage disequilibrium, the sequential Bonferroni correction (Rice 1989) was applied in order to minimize type I errors.

The quantification of genetic diversity in each population was obtained via the computation of observed and expected heterozygosities (H_O and H_E) at each locus, the mean H_O and H_E for each population, and the total number of alleles at each locus and over all loci using POPGENE.

The program POPULATIONS Version 1.2.26 (Langella 1999-2002) was applied to compute Nei's genetic distance, D_A (Nei *et al.* 1983), for all pairwise comparisons and to draw a neighbor-joining tree based on these distances (Takezaki & Nei 1996). Nei's D_A is one of the most commonly used distance measures for microsatellites and is based on the infinite allele model (IAM; Kimura & Crow 1964). For the construction of a tree, Nei's D_A is thought to be superior to many other distance measures regardless of the underlying mutation model (Takezaki & Nei 1996). The trees were displayed and saved as graphic files in the computer application TREEVIEW (Page 1996).

Three different approaches were followed to test for population genetic differentiation: (i) Fisher's exact tests for population differentiation (Raymond & Rousset 1995b; Rousset & Raymond 1997), which evaluate differences in allele and genotype frequencies among populations at each locus, were calculated in GENEPOP (for *a posteriori* significance testing, the sequential Bonferroni correction [Rice 1989] was applied in order to reduce type I errors); (ii) estimates of Wright's fixation index, F_{ST} (Wright 1951, 1965; Weir and Cockerham 1984), were calculated in GENETIX, Version 4.01 (Belkhir 1999); (iii) unbiased multilocus R_{ST} statistics (Slatkin 1995) were estimated with the program RSTCALC (Goodman 1997). Pairwise R_{ST} statistics were also computed among all subspecies represented with a large enough sample size. F_{ST} relies on allele frequencies and is expected to be responsive to recent population dynamics. R_{ST} relies on the stepwise mutation model of microsatellite evolution (SMM; Ohta & Kimura 1973) and is expected to be sensitive to ancient population subdivisions where time was

sufficient for mutations to arise. The significance of F_{ST} and R_{ST} values was evaluated with 10 000 permutations. For all three approaches, the three mitochondrially defined assemblages SW, C, and N (Kryger *et al.* submitted; chapter 4) were treated as populations. In order to rule out false negatives due to the weaker separation between C and N, we also computed the statistics between SW and CN (i.e. C and N combined into one population).

In addition to the traditional way of defining intraspecific nuclear genetic structure by discriminating among populations through allele frequencies and diagnostic alleles, an alternative approach has emerged that considers the individual organism as the ultimate taxon and replaces allele frequencies by the individual multilocus genotype (Vrana & Wheeler 1992; Cornuet *et al.* 1999). Based on the assumption that individuals will have more similar genotypes when they originate from the same group, several methods have been developed to assign individuals to their most likely source population (Paetkau *et al.* 1995; Favre *et al.* 1997; Rannala & Mountain 1997). In this study, assignment tests were performed under the likelihood-based Bayesian method as described by Rannala & Mountain (1997) and implemented in the program GENECLASS (Cornuet *et al.* 1999). Under the conjecture of an equal prior probability density to the allelic frequencies of each locus in each population, the marginal probability of observing an individual with genotype $A_kA_{k'}$ at locus j in population i was shown to equal

$$(n_{ijk} + 1/K_j + 1)\ (n_{ijk} + 1/K_j)/(n_{ij} + 2)\ (n_{ij} + 1), \text{ if } k = k'$$

and

$$2\ (n_{ijk} + 1/K_j)\ (n_{ijk} + 1/K_j)/(n_{ij} + 2)\ (n_{ij} + 1), \text{ if } k \neq k'$$

where n_{ijk} is the number of alleles k sampled at locus j in population i, n_{ij} is the number of gene copies sampled at locus j in population i, and K_j is the total number of alleles observed over all populations at locus j (Rannala & Mountain 1997). Furthermore, a distance-based method of direct assignment was performed in GENECLASS using Nei's standard D (Nei 1972) and Nei's DA. All three approaches of computing genotype assignments were applied under both the "as-is" and the "leave-one-out" procedure.

To test for the amount of biparentally mediated gene flow, coalescence theory derived maximum likelihood estimates for the current migration rates (Beerli & Felsenstein 2001) among the different populations were calculated based on the microsatellite data. This was done using a Markov chain Monte Carlo approach as implemented in MIGRATE Version 1.1 (Beerli 1997-2001). The MIGRATE estimates for theta (per site) were interpreted as indicators of the extant effective population size by calculating $N_e = \theta/4\mu$, with μ as the mutation rate per site per generation.

5.4 Results

Mitochondrial DNA

Haplotype network and nested design. Figure 2 depicts the nested design for the CR-I haplotypes found in *L. saxatilis* following the rules from Templeton *et al.* (1987) and Templeton & Sing (1993). The maximum number of mutational steps for haplotype connections that were confirmed to be parsimonious with a probability $P > 0.95$ was seven. It was not possible to determine a single root for the entire cladogram. There were three major disjoint portions that could not be connected at a 95% parsimony level (clades 4-1, 4-2, and 5-2). Haplotype 57 was separated from all other haplotypes by a large number of mutational steps (from 9 to 20), and its exact interconnection remained uncertain. Within clade 4-2, three disjoint sub-units were retrieved and the connections between them were not parsimonious at a 95% level (dotted lines in Fig. 5.2); 11 mutational steps separated HT20 from HT33, and nine mutational steps separated HT12 from HT35.

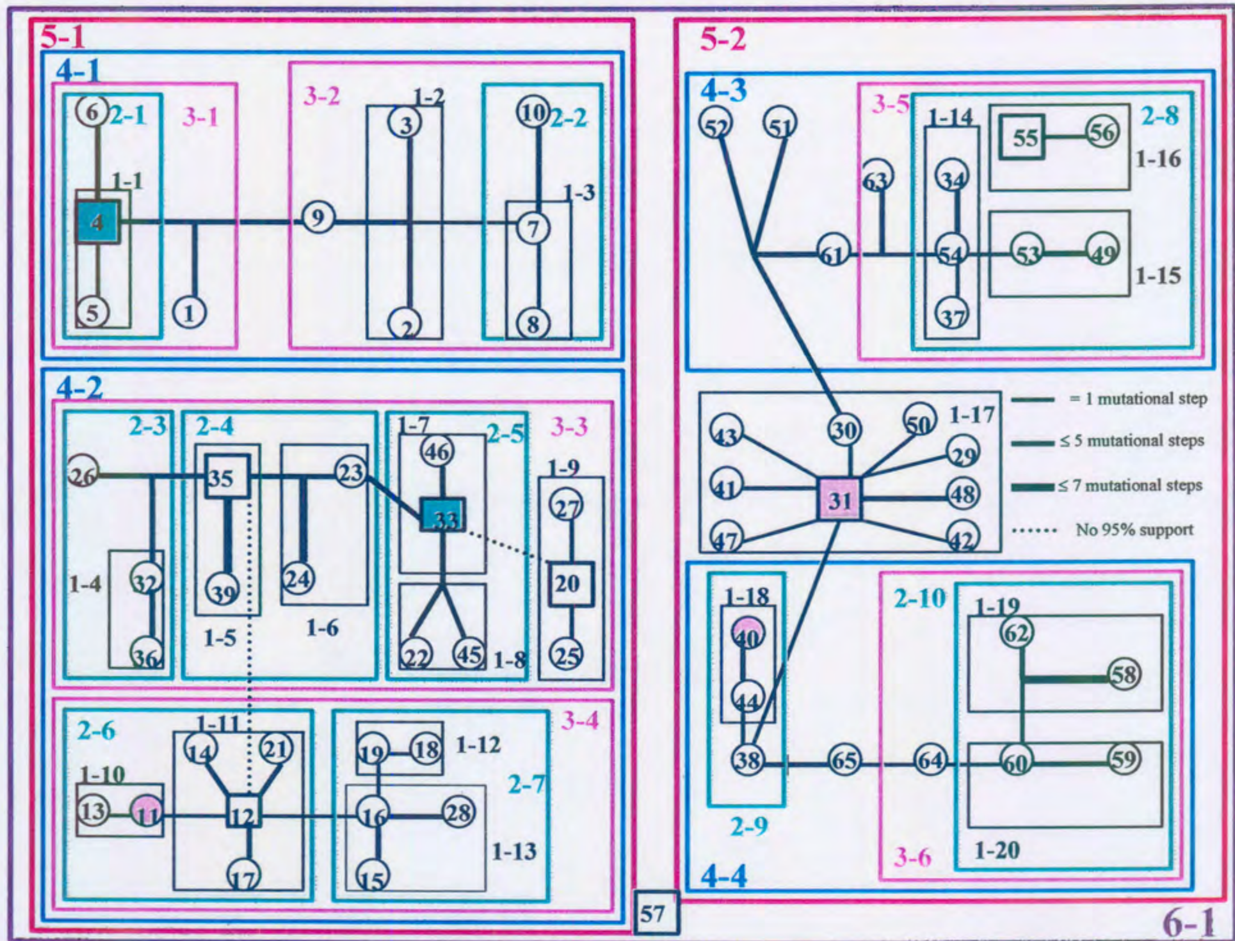


Fig. 5.2 Nested cladogram for the mitochondrial CR-I haplotypes of *Lepus saxatilis* based on a haplotype network constructed with TCS (Clement *et al.* 2000) using the criterion of 95% parsimony (see text for details). Haplotype designations follow Fig. 5.1. Ancestral haplotypes as suggested by TCS are symbolized by squares. Haplotypes with green background occurred 5 times and in different localities, while haplotypes with pink background occurred more than ten times and in different localities. The thickness of the lines indicates the number of mutational steps that interconnect two haplotypes. Clade 4-1 coincided with the SW assemblage identified in the NJ analysis, clade 4-2 corresponded to the C group, and clade 5-2 to the N cluster, respectively.

Permutation analyses of the nested clades. Significant associations of clades and sampling locations were revealed at all clade levels (probability values in bold type in Table 5.1). The contingency analysis indicated strong associations between clades and geographical locations for two 1-step clades (1-7 and 1-17; Table 5.1), three 2-step clades (2-4, 2-6, and 2-9; Table 5.1), three 3-step clades (3-2, 3-3, and 3-4; Table 5.1), two 4-step clades (4-1 and 4-2; Table 5.1), two 5-step clades (5-1 and 5-2; Table 5.1), and for the entire cladogram.

Table 5.1 Nested contingency analysis of geographic associations. Only clades with genetic/geographic variation are testable. The permutational chi-square probabilities were calculated by 10 000 random permutations of the lower level clade categories within the nesting clade *versus* the geographical locality. Significant probabilities are in bold type.

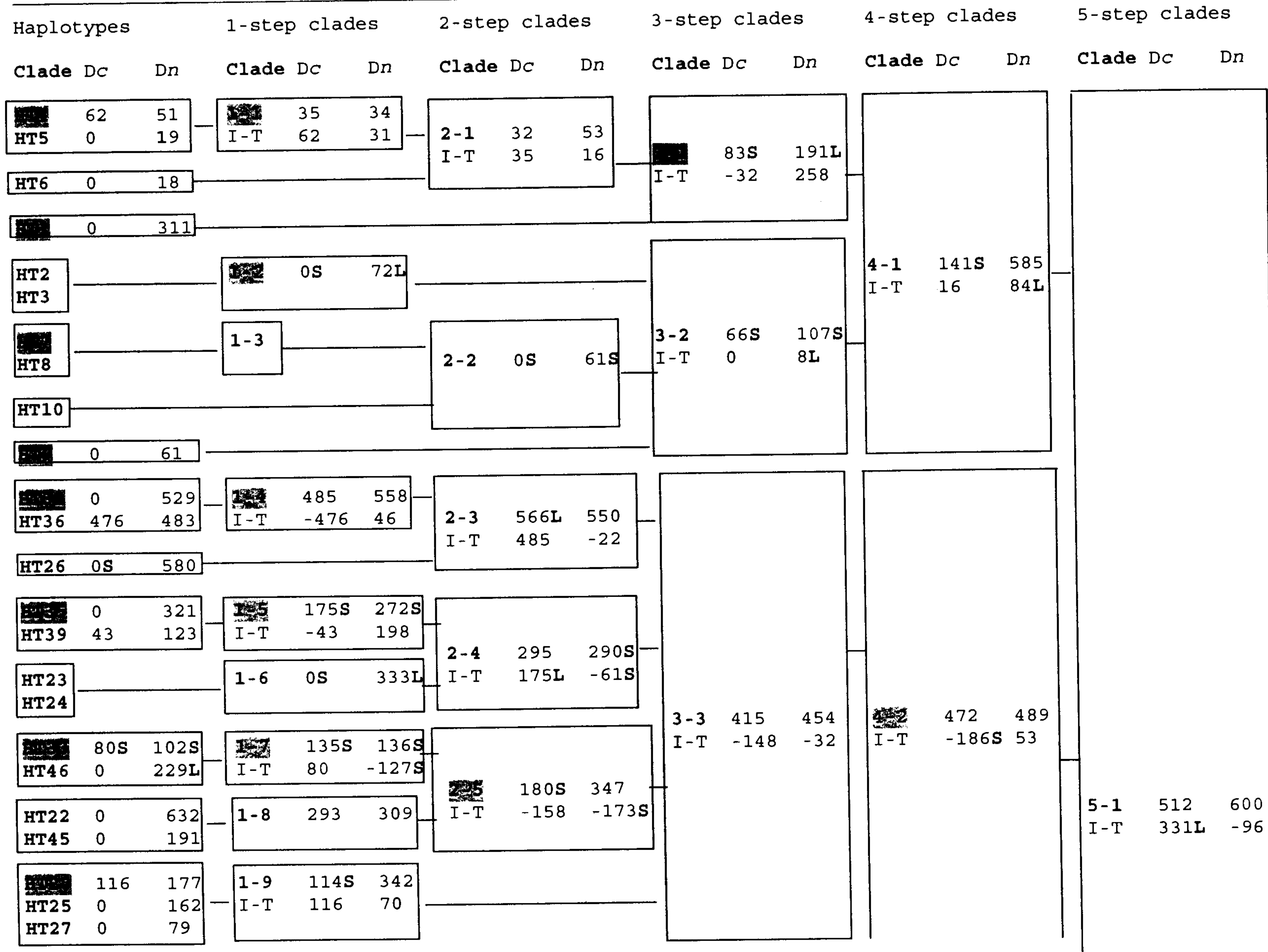
Clade	Permutational χ^2 statistic	Probability
1-1	1.11	1.000
1-4	4.00	1.000
1-5	6.00	0.206
1-7	7.00	**0.047**
1-8	3.00	0.335
1-9	6.67	0.146
1-10	0.78	1.000
1-11	10.50	0.171
1-13	5.00	0.199
1-14	3.00	1.000
1-15	3.00	0.327
1-16	2.00	1.000
1-17	88.94	**0.042**
1-18	15.00	0.068
2-1	0.11	1.000
2-3	7.00	0.142
2-4	11.00	**0.008**
2-5	5.24	0.324
2-6	12.31	**0.002**
2-7	0.69	1.000
2-8	13.33	0.086
2-9	14.40	**0.025**
3-1	12.00	0.167
3-2	16.00	**0.000**
3-3	56.98	**0.000**
3-4	14.79	**0.002**
3-5	10.00	0.117
4-1	24.50	**0.000**
4-2	54.65	**0.000**
4-3	8.67	1.000
4-4	25.00	0.120
5-1	84.83	**0.000**
5-2	73.38	**0.000**
6-1, Entire cladogram	286.68	**0.000**

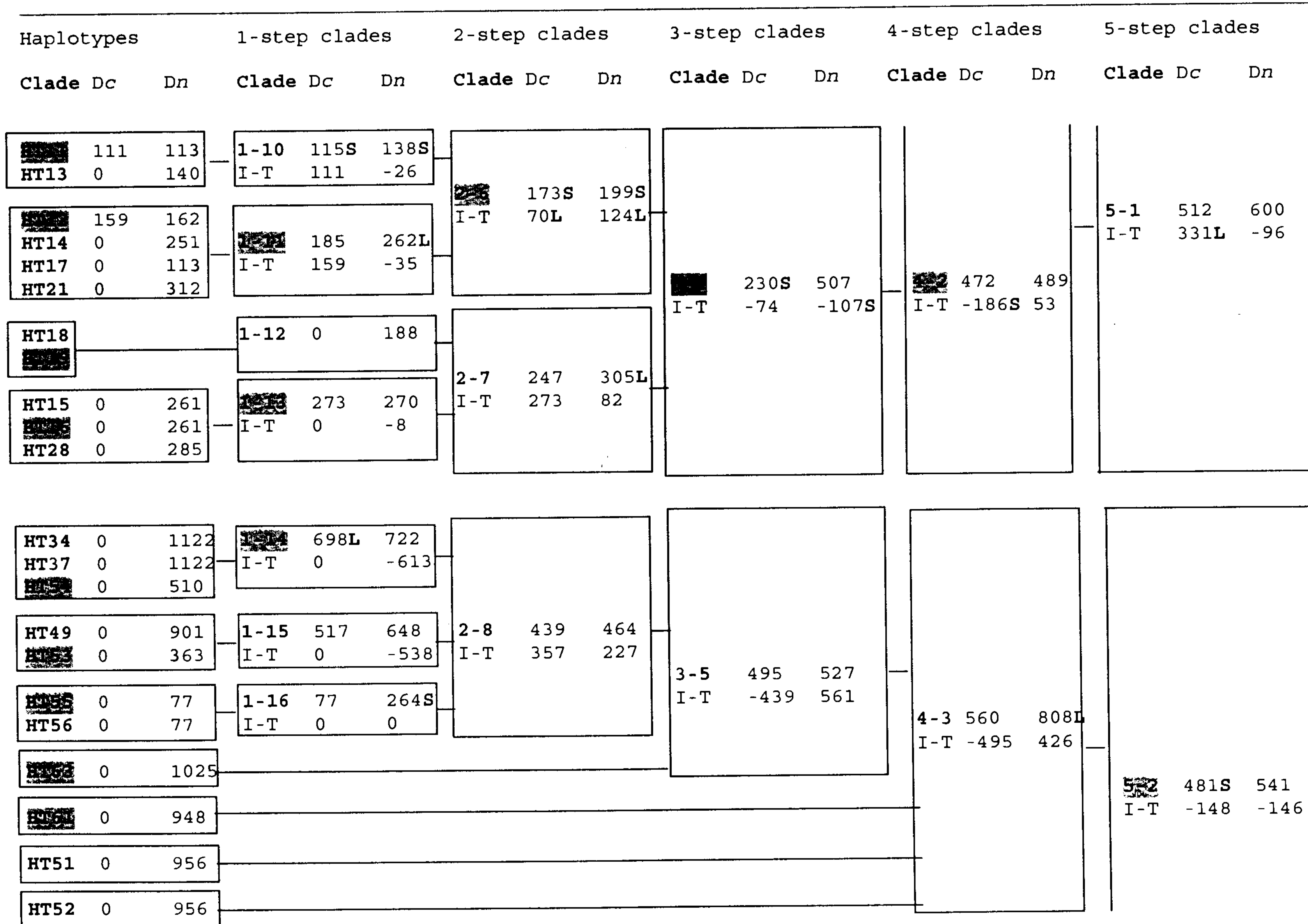
Geographic distance analysis. The analysis of the geographic distances resulted in significant differences for numerous clade (D*c*) and nested clade (D*n*) distances (Fig. 5.3). The interpretations of these results using the latest NCA key inference (Materials and methods) based on Templeton *et al.* (1995) and Crandall & Templeton (1996) are summarized in Table 5.2. At the 1-step level only two of 14 tests were significant , and six of eight tests allowed the usage of the inference key at the 2-step level (Table 5.2 and Fig. 5.3). Three results each were significant at the 3-step and 4-step levels, and all nested clades tested at the 5-step and 6-step level were informative (Table 5.2 and Fig. 5.3). This pattern of increasing statistical power with rising nesting level is often observed in NCA studies (Templeton *et al.* 1995; Alexandrino *et al.* 2002; Branco *et al.* 2002) and suggests that more phylogeographic information can be extracted from higher level clades (Durand *et al.* 1999). The lack of resolution at the lower nesting levels is probably caused by the smaller numbers of observations and lower levels of haplotype variation at these lower levels due to their relatively more recent origin (Crandall & Templeton 1996). Overall, the NCA of mtDNA CR-I haplotypes of the South African scrub hares detected substantial geographical associations and the null hypothesis of no association between the position of a haplotype in the cladogram and its geographical position could clearly be rejected for the species as a whole (Table 5.1 and 5.2; Fig. 5.3).

The inference chain indicated contiguous range expansion (CRE) for eight of the 17 tested clades (Table 5.2). For three clades the geographic sampling scheme was not adequate to distinguish range expansion from fragmentation (FRA)/isolation by distance (IBD) or long distance colonization (LDC), respectively (Table 5.2). The inference chains of two clades at the 2-step level suggested restricted gene flow with isolation by distance (RGF with IBD) as the evolutionary process responsible for the observed associations (Table 5.2). In three instances, past fragmentation (FRA) was identified as the historical event having led to the present partitioning of genetic variation (Table 5.2). The result for one clade was inconclusive.

Table 5.2 Inference chain for the results of the geographical distance analyses. Clade designations follow Figs. 5.2 and 5.3.

Clade	Chain of inference	Inference
1-7	1-2-11-12-NO	contiguous range expansion
1-17	1-2-11-12-NO	contiguous range expansion
2-3	1-2-3-4-9-NO	past fragmentation, (additional support as clades mutationally connected by larger than average number of steps)
2-4	1-2-3-5-15-NO	past fragmentation
2-5	1-2-11-12-NO	contiguous range expansion
2-6	1-2-3-4-NO	restricted gene flow with isolation by distance (additional support by increasing D_C values with increasing clade level)
2-8	1-2-11-17-NO	inconclusive outcome
2-9	1-2-3-4-NO	restricted gene flow with isolation by distance (additional support by increasing D_C values with increasing clade level)
3-2	1-2-3-5-15-16-18-NO	geographic sampling scheme inadequate to discriminate between fragmentation, range expansion, and isolation by distance
3-3	1-2-11-12-NO	contiguous range expansion
3-4	1-2-11-12-NO	contiguous range expansion
4-1	1-2-11-12-13-14-NO	sampling design inadequate to discriminate between contiguous range expansion and long distance colonization
4-2	1-2-11-12-NO	contiguous range expansion
4-4	1-2-3-5-15-NO	past fragmentation, (additional support as clades mutationally connected by larger than average number of steps)
5-1	1-2-3-4-9-10-NO	geographic sampling scheme inadequate to discriminate between fragmentation and isolation by distance
5-2	1-2-11-12-NO	contiguous range expansion
6-1	1-2-11-12-NO	contiguous range expansion


Haplotypes
1-step clades
2-step clades
3-step clades
4-step clades
5-step clades
Clade Dc Dn
Clade Dc Dn
Clade Dc Dn
Clade Dc Dn
Clade Dc Dn
Clade Dc Dn
62 51
HT5 0 19
HT6 0 18
0 311
HT2
HT3
HT8
HT10
0 61
0 529
HT36 476 483
HT26 0S 580
0 321
HT39 43 123
HT23
HT24
80S 102S
HT46 0 229L
HT22 0 632
HT45 0 191
116 177
HT25 0 162
HT27 0 79
35 34
I-T 62 31
0S 72L
1-3
485 558
I-T -476 46
175S 272S
I-T -43 198
1-6 0S 333L
135S 136S
I-T 80 -127S
1-8 293 309
1-9 114S 342
I-T 116 70
2-1 32 53
I-T 35 16
2-2 0S 61S
2-3 566L 550
I-T 485 -22
2-4 295 290S
I-T 175L -61S
180S 347
I-T -158 -173S
83S 191L
I-T -32 258
3-2 66S 107S
I-T 0 8L
3-3 415 454
I-T -148 -32
4-1 141S 585
I-T 16 84L
472 489
I-T -186S 53
5-1 512 600
I-T 331L -96


Haplotypes
1-step clades
2-step clades
3-step clades
4-step clades
5-step clades
Clade Dc Dn
111 113
HT13 0 140
159 162
HT14 0 251
HT17 0 113
HT21 0 312
HT18
HT15 0 261
0 261
HT28 0 285
HT34 0 1122
HT37 0 1122
0 510
HT49 0 901
0 363
0 77
HT56 0 77
0 1025
0 948
HT51 0 956
HT52 0 956
1-10 115S 138S
I-T 111 -26
185 262L
I-T 159 -35
1-12 0 188
273 270
I-T 0 -8
698L 722
I-T 0 -613
1-15 517 648
I-T 0 -538
1-16 77 264S
I-T 0 0
173S 199S
I-T 70L 124L
2-7 247 305L
I-T 273 82
2-8 439 464
I-T 357 227
230S 507
I-T -74 -107S
3-5 495 527
I-T -439 561
472 489
I-T -186S 53
4-3 560 808L
I-T -495 426
5-1 512 600
I-T 331L -96
481S 541
I-T -148 -146

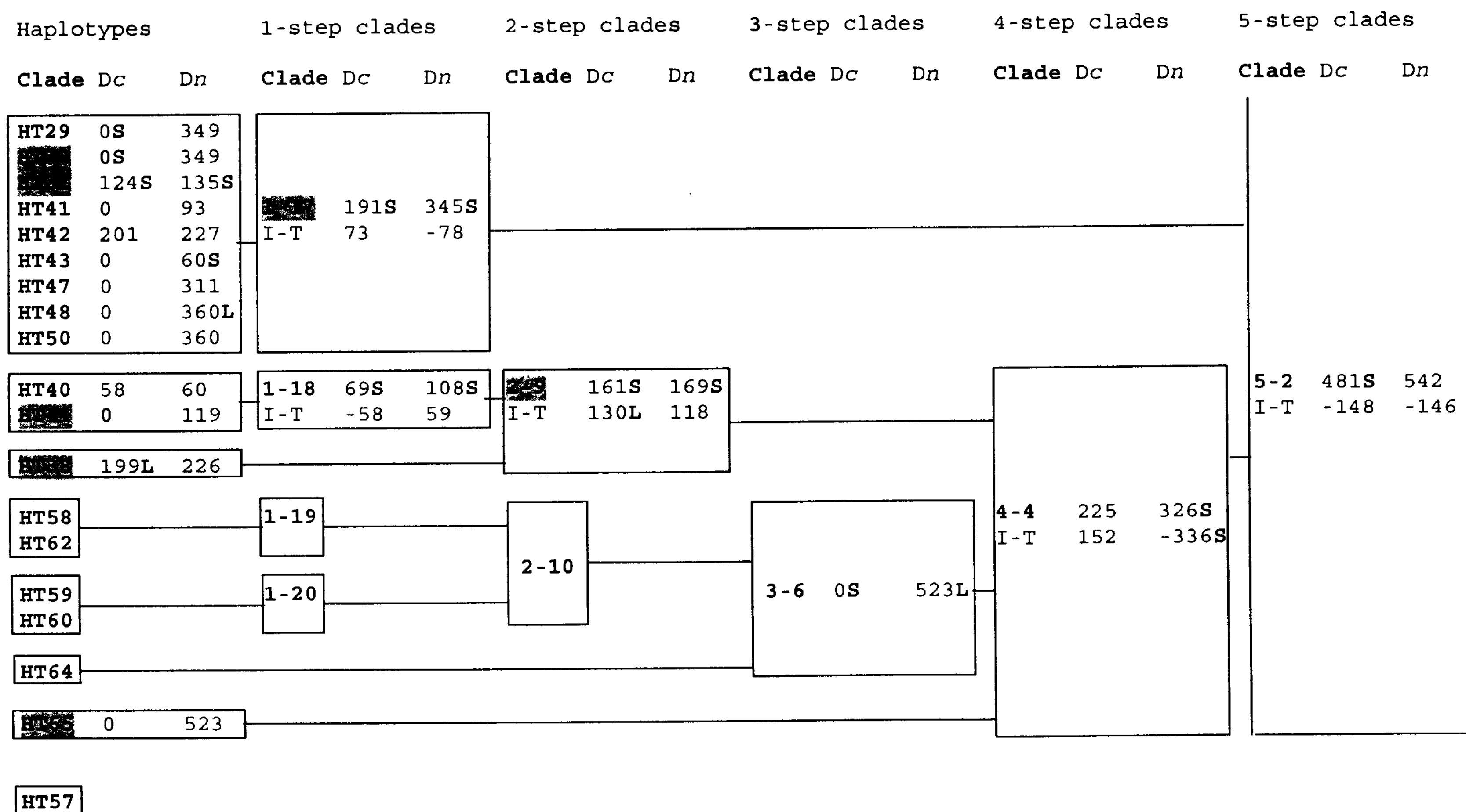


Fig. 5.3 Results of the nested geographic analysis of the scrub hare CR-I haplotypes. Haplotype designations follow Fig. 5.1. The nested design with the clade designations is given in Fig. 5.2. Clade (D*c*) and nested clade (D*n*) distances are given to the right of each haplotype or clade. Interior clades are shaded, interior *vs* tip contrasts for D*c* and D*n* are indicated as "I-T" in the corresponding nesting clade. The bold type letters "S" and "L" indicate that the distance is significantly small or large, respectively, at the 5% level.

Microsatellites

HWE and genotypic disequilibrium. In the case of the SW population, all observed genotype distributions were in HWE (Table A16 in Appendix), despite the small sample size. For populations C and N, only one locus each showed a significant ($P < 0.01$) deviation from HWE (Lsa3 and Lsa4 respectively; Table A16 in Appendix). Both departures from HWE were due to heterozygote deficits, which may have resulted from the presence of null alleles or substructured populations (Wahlund effect). Since no deviation from HWE was found at any one locus in more than one population (data not shown), all loci were considered to be selectively neutral and were maintained for further analyses. After Bonferroni corrections only one of 45 exact tests for pairwise linkage disequilibrium between the six loci was significant at the $P \leq 0.01$ level (Lsa2 and Lsa3 in population C; Table A17 in Appendix). This is less than the expected incidence of type I errors (2.25 expected spurious significant results by chance fluctuation alone at $P < 0.05$). Independent segregation of alleles at the six loci under investigation was therefore inferred and all loci were retained for subsequent analyses.

Allelic variation of microsatellites. The analysis of six microsatellite loci yielded 40 alleles in a sample of 156 scrub hares (three samples did not amplify consistently and were therefore omitted; for compound genotypes see Appendix Table A9). All loci were polymorphic in each subpopulation (Appendix Tables A10-A15). The most variable locus was Lsa2 with nine alleles detected for the whole sample; the least variable locus was Lsa4 with four alleles. The total number of alleles per locus per population ranged from three to nine. The mean number of alleles was lowest in SW (4.17) and highest in N (6.17). Locus-specific observed and expected heterozygosities (H_O/H_E) were lowest at Lsa4 (0.20/0.26) and highest at Lsa1 (0.74/0.69; Table 5.3). The population-specific mean H_O values ranged from 0.44-0.52 while mean H_E extended from 0.50-0.53 (data not shown). The frequency of the most common allele for each of the six loci varied between 0.33 and 0.92 (Appendix Tables A10-A15). Three private alleles were found in the C group (at loci Lsa6 and Lsa8), and five in the N subpopulation (one each at loci Lsa1-4

and Lsa6). All private alleles occurred with low frequencies (all ≤0.0682). No private alleles were found for the SW assemblage, possibly due to the smaller sample size.

Population structure. Only two of six loci (Lsa1 and Lsa8) revealed significant differences in allele and genotype frequencies between SW and C and between SW and CN (Table 5.3). The same two loci showed a significant difference in genotype frequencies between SW and N, but only Lsa1 also indicated a significant difference in allele frequencies between these two assemblages (Table 5.3). The results of the Fisher's exact tests did not allow rejection of the null hypothesis of uniform allelic and genotypic frequencies for any of the pairwise population comparisons at three of the six loci (Table 5.3). Lsa6 exhibited a significant difference in allele and genotype frequencies in the comparison between C and N (Table 5.3).

Table 5.3 Results of Fisher's exact tests for genic/genotypic differentiation among the three scrub hare assemblages (SW = southwestern, C = central, N = northern, and CN = central-northern); * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, n.s. = not significant. Also presented are the expected and observed heterozygosities (H_E & H_O) for each locus.

Locus	SW - C	SW – N	SW-CN	C - N	H_E	H_O
Lsa1	*/*	***/***	***/***	n.s./n.s.	0.69	0.74
Lsa2	n.s./n.s.	n.s./n.s.	n.s./n.s.	n.s./n.s.	0.59	0.56
Lsa3	n.s./n.s.	n.s./n.s.	n.s./n.s.	n.s./n.s.	0.68	0.50
Lsa4	n.s./n.s.	n.s./n.s.	n.s./n.s.	n.s./n.s.	0.26	0.20
Lsa6	n.s./n.s.	n.s./n.s.	n.s./n.s.	***/**	0.24	0.22
Lsa8	***/***	n.s./*	***/**	n.s./n.s.	0.66	0.68

Table 5.4 shows the proportion of the genetic variation attributable to differences between populations quantified with F_{ST} and R_{ST} statistics. All values were relatively low and indicative of only moderate subdivisions. Yet, the F_{ST} calculations revealed a substantial degree of differentiation (F_{ST} values significantly greater than zero and larger than 0.05) for the global analysis on the total sample and for the pairwise comparisons between SW and C, between C and N, and between SW and CN. The R_{ST} analyses reflected a significant extent of genetic substructuring in the global test as well as between the populations SW and C and between SW and CN. In contrast to the F_{ST}

results, the R_{ST} value for the comparison between C and N was not significantly greater than zero, but the comparison between SW and N retrieved a significant differentiation (Table 5.4). The R_{ST} values were in general lower than the F_{ST} values, with the exception of the comparison between SW and N, where this trend was reversed.

Table 5.4 Total and pairwise F_{ST} and R_{ST} values for the three scrub hare populations (SW = southwestern, C = central, N = northern, and CN = central-northern) as calculated by GENETIX and RSTCALC, respectively. Significance levels after 10 000 permutations indicated in parenthesis as * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, n.s. = not significant.

	total	SW-C	SW-N	C-N	SW-CN
F_{ST}	0.0622 (**)	0.0895 (**)	0.0323 (n.s.)	0.0675 (**)	0.0951 (***)
R_{ST}	0.03641 (***)	0.03963 (*)	0.09472 (***)	0.00930 (n.s.)	0.0782 (**)

Genetic differentiation among the populations was also assessed using assignment tests in GENECLASS (Cornuet *et al.* 1999). The comparison of different assignment algorithms demonstrated that assignments based on Nei's D (based on the IAM) outperformed those based on distance measures that rely on the SMM (data not shown), thus concurring with Cornuet *et al.* (1999). Given the interrupted structure of repeat arrays in two of the loci used here, the mutational process is supposedly more similar to the IAM than the SMM.

As reported in the literature (Cornuet *et al.* 1999), the assigment tests retrieved higher scores employing the Bayesian method (62.82% correctly assigned; Table 5.5) than with the distance method (53.85 - 58.33% correctly assigned; Table 5.5) when the assignment was done among a partitioning into three populations. Irrespective of the algorithm used, the "as-is" procedure always outperformed the "leave-one-out" procedure (Table 5.5). We therefore chose the results of assignments obtained with the Bayesian method following the "as-is" procedure (Tables 5.6 and 5.7). The overall probability of correctly assigning animals to their population of origin based on their six-locus composite genotype was 51.92% when treating each sampling location as a distinct population. The score increased to 62.82% under the hypothesis of three populations as defined by the mitochondrial assemblages SW, C, and N, and to 80.13% under the hypothesis of only two source populations, SW against CN (Table 5.5).

Table 5.5 Percentages of overall correct assignment achieved under three different hypotheses: all 23 locations as populations, SW, C, and N as populations, or SW and CN as populations. Analyses were based on three different approaches implemented in GENECLASS - the model-based Bayesian analysis and two distance-based analyses using either Nei's DA or Nei's Standard D. Each of the three analyses was tested both under the "as-is" option (a-i) and under the "leave-one-out" option (l-o-o).

Hypothesis	Bayesian	Bayesian	Nei's DA	Nei's DA	Nei's D	Nei's D
	a-i	l-o-o	a-i	l-o-o	a-i	l-o-o
23 lineages	51.92	13.46	43.59	11.54	44.87	16.03
3 lineages	62.82	50.64	58.33	43.59	53.85	48.72
2 lineages	80.13	73.08	75	69.23	75	71.15

Based on the assumption of three distinct scrub hare populations, 74.07% of the animals collected in the southwestern Cape were correctly assigned to the SW population, but only 62.71% and 57.14% of the individuals collected in the central and northern regions, respectively, were assigned correctly (Table 5.6). Misassignments of animals sampled from the southwestern Cape were fairly equally divided between C (14.8%) and N (11.11%). Likewise, misassignments of hares originating from the central population fell in equal proportions (18.64% each) to SW and N. The majority of the misassigned individuals obtained from northern regions fell within the C group (32.86%; Table 5.6).

Table 5.6 Assignment test results (Bayesian, "as-is") given as percentages for the hypothesis of three distinct lineages according to mitochondrially defined assemblages (SW = southwestern, C = central, and N = northern). The populations of geographic origin are given in the top row, the assigned populations are given in the left column. The diagonal shows the percentage of correct assignments (values in bold); n indicates the sample size.

Population	SW (n = 27)	C (n = 59)	N (n = 70)
SW	**74.07**	18.64	8.57
C	14.8	**62.71**	32.86
N	11.11	18.64	**57.14**

When applying the hypothesis of only two distinct populations of scrub hares in southern Africa, SW and the rest (CN), the percentage of correct assignments rose to approximately 80% for both populations (Table 5.7).

Table 5.7 Assignment test results (Bayesian, "as-is") given as percentages for the hypothesis of two distinct lineages according to the mitochondrially defined assemblages (SW = southwestern and CN = central-northern) in the scrub hare. The populations of geographic origin are given in the top row, the assigned populations are given in the left column. The diagonal in bold shows the percentage of correct assignments, n indicates the sample size.

Lineage	SW (n = 27)	CN (n = 129)
SW	**77.77**	19.38
CN	22.22	**80.62**

The genetic distances Nei's D_A (Nei *et al.* 1983) between the three scrub hare populations showed a more pronounced nuclear genetic distinction between SW and both other populations than between C and N (Table 5.8; for pairwise Nei's D_A values among all sample localities see Appendix, Table A19). The NJ tree derived from Nei's D_A (Fig. 5.4) showed random clustering of the sample localities. There was no correlation between the geographic position of a sample locality and its position in the tree. Generally, the bootstrap support values were very low (under 50%; Fig. 5.4) indicating a lack of phylogenetic resolution based on the six microsatellite loci used here.

Table 5.8 Nuclear genetic distance (Nei's D_A) between the three mtDNA defined scrub hare populations (SW = southwestern, C = central, and N = northern).

	SW	C	N
SW			
C	0.053		
N	0.045	0.021	

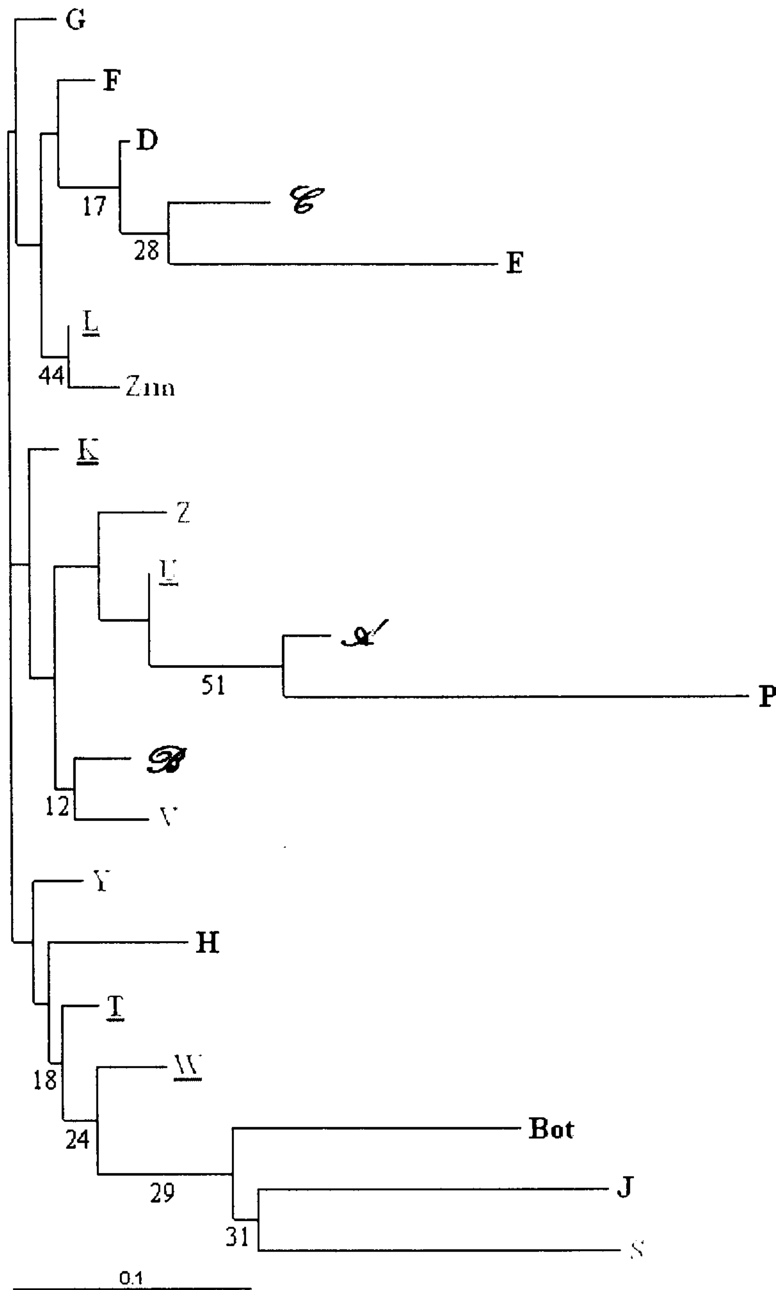


Fig. 5.4 Neighbor-joining tree for 21 scrub hare sample localities based on Nei's D_A (Nei *et al.* 1983) calculated from six microsatellite loci in POPULATIONS (Langella 1999-2002). The values beneath the branches represent the bootstrap support after 1000 replicates. Locality designations follow Fig. 5.1. The letters in "Kunstler Script" font code for sample localities defining the mitochondrial SW assemblage, letters in "Times New Roman" black code for the C cluster, gray codes for the N group (cf. Fig. 5.1), and gray and underlined marks localities with both central and northern haplotypes.

The estimates of the number of migrants between the three scrub hare populations were not homogeneously distributed (Table 5.9). The migration from SW to C and to N was very high. In marked contrast, the movement of animals from C and N into the southwestern area was relatively low (Table 5.8). The greatest movement was registered with ~ 45 migrants per generation from C to N, whereas only 11 animals per generation migrated in the opposite direction.

Estimates of population size, assuming a standard microsatellite mutation rate of 5×10^{-4} per locus per generation (Cornuet *et al.* 1999), were 1026 for SW, 1181 for C, and 2360 for N, respectively. These values were magnitudes smaller than the values for the female population sizes calculated from the mitochondrial sequence data (25 359 for SW, 94 515 for C, and 80 796 for N; Kryger *et al.* submitted; chapter 4). Clearly, they should have been approximately twice as large since the microsatellite data represent the total population sizes (i.e. includes both male and female animals). It therefore seems likely that the six loci used in this study evolve at a slower rate than suggested in Cornuet *et al.* (1999). Mutation rates for the six loci were estimated with the formula $\mu = \theta/4N_e$ and doubling the values of the effective population sizes attained from the mtDNA data. Using this approach the average mutation rate over all loci was estimated at 1.5×10^{-5}, the locus with the lowest mutation rate was Lsa6 (9×10^{-6}), and the locus with the highest mutation rate was Lsa4 (3×10^{-5}).

Table 5.9 Number of migrants per generation and population-size estimator (θ) for the three scrub hare populations (SW = southwestern, C = central, and N = northern) based on six microsatellite loci as computed by MIGRATE. The population of origin is indicated in the top row and the receiving population is shown in the left column.

receiving	SW	C	N	θ
SW		5.2120	6.5021	2.05144
C	20.1015		11.1900	2.36110
N	33.9442	44.7913		4.72004

5.5 Discussion

Mitochondrial DNA NCA

The three disjoint subunits contained in the scrub hare CR-I haplotype network (Fig. 5.2) identified via the TCS analysis corresponded precisely to the three populations retrieved by the NJ analysis of the mtDNA sequences (Kryger *et al.* submitted; chapter 4). Nesting clade 4-1 (Fig. 5.2) was consistent with the SW subpopulation in the NJ tree, nesting clade 4-2 (Fig. 5.2) matched the C group in the NJ tree, and nesting clade 5-2 (Fig. 5.2) corresponded to the N assemblage.

The NCA inference for clade 5-1 could not discern between past fragmentation and isolation by distance as the driving evolutionary force (Table 5.2). In light of the distinctiveness of clade 4-1 as the SW cluster in the NJ analyses, past fragmentation seemed the more likely factor responsible for the observed spatial distribution of genetic variation. Both analyses (NJ and NCA) grouped several haplotypes (HT32, HT33, HT35, HT36, HT39, HT45, and HT46) that had been collected in the northern part of the sample area into the central assemblage/clade 4-2 (Fig. 5.2). The discrepancy between the phylogenetic placement of these haplotypes and their geographic origin was thought to be due to the migration of scrub hares from the central areas to the north (Kryger *et al.* submitted; chapter 4). In contrast, the NCA detected past fragmentation in two of the three subclades containing these haplotypes along with geographically central haplotypes (2-3 and 2-4; Fig. 5.2). This could be the signature of the hypothesized separation of the central from the northern group some 18 000 years ago (Kryger *et al.* submitted; chapter 4) and would consequently represent the retention of ancestral polymorphism in the northern group. However, clade 2-5 (Fig. 5.2) also comprised haplotypes from both geographic areas (C and N), but the NCA inference clearly indicated contiguous range expansion as the underlying process. Consequently, the female mediated gene flow from C to N could not be refuted altogether. It has been emphasized that the various evolutionary forces shaping geographic associations of haplotypes are not mutually exclusive (Templeton *et al.* 1995; Durand *et al.* 1999). In the present case, a pattern of gene flow superimposed on the signal of previously fragmented populations can be

explained by relatively recent vicariance and subsequent secondary contact between the clusters C and N. The difficulties of achieving a clear demarcation of these two clusters in the NJ analysis may simply reflect the intermediate level of phylogenetic separation between them which has not fully developed into reciprocal monophyly since the isolation did not last for a sufficient number of generations (Avise 2000).

The haplotype network contained one pronounced star-like phylogeny with clade 1-17 in the centre of clade 5-2 (Fig. 5.2). The central haplotype (HT31) was shared by 14 individuals from seven different sampling localities with a geographic range in excess of 700 km (data not shown; see chapter 4) and was identified as being ancestral by the TCS analysis (square symbol for haplotype 31 in Fig. 5.2). According to Avise (2000), such a star-like pattern with a common haplotype in the centre connected via independent mutational steps with numerous rare haplotypes at the tips can be regarded as a signal of a recent population size expansion from a small number of founder individuals. This finding was consistent with the result of the mismatch distribution analysis (Kryger *et al.* submitted; chapter 4) where the northern assemblage of scrub hares (corresponding to clade 5-2 in the NCA, Fig. 5.2) presumably experienced a sudden population size expansion 45 684 - 52 083 years ago. This was further corroborated by the NCA inference of contiguous range expansion (CRE) for clades 1-17 and 5-2 (Table 5.2; Fig. 5.3).

For clade 4-1 (Fig. 5.2; corresponding to the SW population) and clade 4-2 (Fig. 5.2; corresponding to the C population), the TCS constructed haplotype network did not reveal strong evidence for a rapid size expansion. Nonetheless, the predominant role in shaping the present genetic structure in clade 4-2 and the three subclades 3-3, 3-4, and 2-5 was played by CRE, which corroborated the scenario of a population expansion for population C. For clade 2-6 (a nested clade within 4-2 comprising haplotypes from Oudtshoorn, Grahamstown and Tussen-die-Riviere) the main factor shaping the geographic distribution of genetic variation was recurrent gene flow with isolation by distance. This confirmed the genetic cohesion of these areas (and therefore also the C group) as one population. The complex mixture of evolutionary dynamics impacting on clade 4-2 was probably responsible for blurring the signal of population expansion. The mismatch analysis (Kryger *et al.* submitted; chapter 4) similarly detected a weaker signal

of size expansion in the central assemblage, but the distribution was clearly unimodal for the southwestern group. It therefore seemed probable that the small sample size (27 animals) was responsible for the lack of a star-shaped phylogeny within the TCS-derived haplotype network for SW/clade 4-1. This was substantiated by the NCA which could not distinguish between contiguous range expansion and long distance colonization as an explanation for the observed geographic association of haplotypes.

The NCA highlighted an allopatric fragmentation event within clade 4-4 (Fig. 5.2; Table 5.2) resulting in the separation of clade 2-9 from 3-6. A potential barrier to gene flow between the animals from Zimbabwe (clade 3-6) and those originating from Pretoria, Rustenburg, Vaalwater, and Vryburg (clade 2-9; all in the northern parts of South Africa) could be the Limpopo River basin that separates South Africa from Zimbabwe. Meester (1958) and Bloomer & Robinson (unpublished data) suggested this basin as a barrier to dispersal for *Myosorex* (a shrew species) and *Heterohyrax brucei* (yellowspotted hyrax), respectively. Both species prefer humid, high-lying habitats and are presumably not capable of dispersing through the arid, low-lying river basin. The scrub hare also shows a tendency to avoid arid habitats and is, in fact, replaced in such areas by the Cape hare. Alternatively, it is feasible that the Limpopo River experienced recurrent episodes of drying out alternating with periods of high water levels during the Pleistocene and Holocene climatic oscillations. Scrub hares could have dispersed across the river bed in arid times. In pluvial periods, however, the river may have functioned as a permanent barrier resulting in the disruption of localized gene flow. In a period of extended isolation it is conceivable that the fragmented scrub hare populations might have adapted to the dryer conditions north of the Limpopo, and to the more mesic conditions that existed south of the river.

Heterozygosity and mutation rates of microsatellite loci

Jarne & Lagoda (1996) reported that the average expected heterozygosity in microsatellites is usually between 50% and 100%. In the present study values were only slightly higher than 50%. A possible explanation may be that two of the six loci comprised interrupted repeat motifs. Interruptions in the core sequence seem to stabilize

the array of repeats, leading to these interrupted microsatellites being less variable than pure repeats (Jarne & Lagoda 1996; Ellegren 2000). Relatively low levels of heterozygosity can also be the result of null alleles (= non-amplifying alleles) that arise from point mutations at primer binding sites, thus preventing primers from annealing (Callen *et al.* 1993). The presence of null alleles was suspected for two loci in this study, Lsa3 and Lsa4 (see Results). However, microsatellite based studies on the European rabbit by Surridge *et al.* (1999a, b) found comparable levels of heterozygosity (ranging from 14% to 71%, but most frequently at 50%). Andersson *et al.* (1999), who applied rabbit microsatellite primers to European and mountian hares, also obtained heterozygosity values that ranged from 11% to 73%, with an average of approximately 42%.

The average estimated mutation rate over all six loci used in this study was 1.5×10^{-5} per locus per generation and thus approximately seven times lower than the rate determined for human microsatellites (1×10^{-4}; Edwards *et al.* 1992; Weber & Wong 1993) and about four times lower than the value quoted at porcine microsatellite loci (6×10^{-5}; Ellegren 1995). This could be due to the relatively low number of repeat units in the loci used here. However, the value was larger than the rate estimated for microsatellite loci in *Drosophila melanogaster* (6.3×10^{-6}; Schug *et al.* 1997). It must be emphasized that in order to obtain precise estimates for mutation rates of microsatellite loci, it is necessary to observe mutation events over consecutive generations from known parents. In general, five mutation events within the same geneological line per locus would be required to get an accurate estimate of the mutation rate (Estoup & Cornuet 1999); such an experimental procedure was beyond the scope of this study.

Population structure inferred from microsatellites

The results of the Fisher's exact tests (Table 5.3) revealed significant genetic structure among the populations SW and C, SW and N, and SW and CN for two loci (Lsa1 and Lsa8). The lack of structure retrieved with Lsa3 and Lsa4 may be due to homoplasy or null alleles (as suggested by the departures from HWE in both loci). Locus Lsa6, despite

having the lowest heterozygosity values, was the only locus to detect a significant genetic partitioning between populations C and N (Table 5.3).

The F_{ST} statistics indicated the presence of moderate genetic differentiation (values > 0.05; Table 5.4) for the entire sample and among the populations SW and C, C and N, and SW and CN. The R_{ST} values suggested significant but rather low genetic differentiation (values < 0.05) for the entire sample and between assemblages SW and C and moderate structuring of genetic variation among populations SW and N, and SW and CN (Table 5.4). Gaggiotti *et al.* (1999) found that R_{ST} performs better with large sample sizes (n > 50) and many loci (n > 20) whereas F_{ST} is more reliable when sample sizes are moderate to small (n < 10) and the number of scored loci is low (n < 20). In this study, sample sizes were moderate to large (in all instances n > 10) and the number of scored loci low (n = 6). This may account for the F_{ST} values being higher than the R_{ST} values. The higher sensitivity of F_{ST} for the detection of the genetic differentiation between groups C and N (Table 5.4) could be due to the relatively recent date of their separation. The higher sensitivity of R_{ST} for the recognition of a significant subdivision between SW and N possibly resulted from the more ancient subdivision between these two groups.

The results of the assignment tests confirmed the presence of genetic subdivision among the three scrub hare populations. A substantial proportion of animals were assigned to the correct source population (Tables 5.5-5.7). The fact that the proportion of correct assignments was higher for the test based on three lineages compared to the test based on 23 lineages (Table 5.5) supported the validity of the population subdivision into SW, C and N. A closer investigation of the actual pattern of misassignments (Table 5.6) underpinned the relative distinctiveness of the SW population with less than 15% of genotypes misplaced into C or N. In contrast, more ambiguities were encountered when attempting to distinguish between the central or northern origin of a genotype. Almost 33% of northern animals were misassigned to the central population. These individuals did not coincide with the individuals collected in the north but carrying central mtDNA haplotypes (Fig. 5.1). The dramatic improvement of assignment accuracy when pooling C and N into one combined population (CN) and juxtaposing it to the SW group (Table 5.7) suggested that the microsatellite genotypic variation between C and N was minimal. The

values of Nei's D_S (Table 5.8) similarly indicated that the nuclear genetic distinction between C and N was much weaker than between SW and either C or N.

Discordance among marker sets

Sample locations clearly belonging to the same mtDNA-lineage did not cluster together in the microsatellite derived NJ tree (Fig. 5.4). The topology remained unresolved when excluding those animals that were misassigned or when treating single individuals as operational taxonomic units (data not shown). This lack of structure in the microsatellite derived phylogeny could be due to several factors. First, relatively few microsatellite loci were used in the current study, consequently the resolution power may not have been sufficient to recover the correct topology (Takezaki & Nei 1996). Secondly, it is possible that the high mutation rate of autosomal microsatellite markers can lead to underestimations of genetic differentiation (Balloux *et al.* 2000). Under the stepwise mutation model (SMM; Ohta & Kimura 1973), a fair amount of homoplasy (co-occurrence of alleles that are identical in state but not by descent) is to be expected in microsatellites (Estoup *et al.* 1995b). Furthermore, size homoplasy (where one electromorph actually consists of several different alleles) has been shown to occur mostly among populations, rather than within (Viard *et al.* 1998), thus influencing the detection of population structure negatively.

A third, biological explanation for the incongruence between the two marker sets could be male-biased dispersal combined with female philopatry (see Avise 1995, 2000). Several studies on lagomorphs have observed that natal dispersal is biased in favor of males (Bell & Webb 1991; Webb *et al.* 1995; Surridge *et al.* 1999b for the European rabbit; Reitz & Leonard 1994 for the European hare). If scrub hares follow this general trend and females remain close to their natal home range throughout their lives, maternally inherited genetic variation could well be partitioned geographically in this species. Yet, those partitions may not necessarily represent salient intraspecific units that will also be registered by biparental markers (Avise 2000). Deeper topologies in multiple DNA transmission pathways are congruent with one another after reproductive connections have been severed for a substantial period. Usually this process takes

considerably more time measured in generations than numbers of effective breeders (Avise 2000). With effective female populations sizes between 25 359 and 94 515 (Kryger *et al.* submitted; chapter 4) and a generation time in hares of two years (Marboutin & Peroux 1995), the timeframe necessary for attaining congruence of deep topologies in maternally and biparentally mediated gene trees might range from 101 436 to 378 060 years. The separations between the scrub hare populations presumably only date back 18 080 to 61 235 years (Kryger *et al.* submitted; chapter 4).

The hypothesis of male-biased dispersal in scrub hares was corroborated by the comparison of migration rates calculated from the two different marker sets. The number of migrants per generation estimated from the microsatellite data was much higher than one in all six directions (Table 5.9) and thus strongly contrasted the lack of female-mediated gene flow between SW and the other two groups derived from the mitochondrial data (Kryger *et al.* submitted; chapter 4). Furthermore, the number of migrants per generation between C and N was drastically higher when inferred from micorsatellites than from the mitochondrial CR-I data (11.19 animals in comparison to 0.918 females from N to C, and 44.7913 animals in comparison to 1.8502 females from C to N; Table 5.8 and chapter 4). Based on the assumption of equal sex ratios and the equal contribution of both sexes to reproduction, the number of migrants estimated from mtDNA is expected to be only half that estimated from biparentally inherited microsatellites (Girman *et al.* 2001). The fact that the number of migrants estimated from the microsatellite data was between 10-24 times the number calculated from mtDNA strongly supported the conclusion of male-biased interpopulation dispersal in scrub hares.

Phylogeographic breaks and concordance with other small mammals

The mtDNA CR-I gene tree of *L. saxatilis* from southern Africa was characterized by a deep division with large genetic gaps (phylogeographic category I; Avise *et al.* 1987; Avise 2000), whereas the microsatellite NJ tree was shallow without any major genetic gaps (phylogeographic category IV or V; Avise *et al.* 1987; Avise 2000). However, allele frequency analyses of the microsatellites detected significant genetic gaps in the nuclear variation between the phylogroups identified via the mtDNA analyses, and thus also

indicated the phylogeographic category I for the scrub hare. A category I pattern is a common result for small- to medium-bodied terrestrial mammals (Avise 2000) and has been reported for the European rabbit on the Iberian peninsula (Biju-Duval *et al.* 1991) as well as for the southern African rock hyrax (Prinsloo & Robinson 1992), both based on mtDNA.

The most prominent genetic discontinuety distinguished the SW population as a deeply allopatric lineage from the remaining *L. saxatilis* and was indicative of a long-term population isolation and divergence. No immediately obvious long-term extrinsic impediment to gene flow could be identified as an explanation for this phylogeographic discontinuity. But the geographic area occupied by this lineage is congruent with the South African "mediterranean climatic zone" (Walton 1984) that is characterized by hot, dry summers and cool winters. Carcasson (1964) has defined the same geographical area as the zoochorological Cape zone. This break has not been found in other South African small mammals such as the rock hyrax (Prinsloo & Robinson 1992), Smith's red rock rabbit (Matthee & Robinson 1996), the yellow mongoose (Jansen vanVuuren & Robinson 1997), or the springhare (Matthee & Robinson 1997). The rock hyrax has a Pliocenic origin and is much older than the scrub hare, which may account for the different pattern. Furthermore, it is possible that differences in habitat preference may underlie the lack of complementarity. Rock hyrax and Smith's red rock rabbit are both rock dwelling small mammals. The scrub hare on the other hand, contrary to initial habitat descriptions (Shortridge 1934) and the naming of the species ("saxatilis" indicating a preference for rocky habitat), is not confined to rocky areas but occupies a much broader range of habitats (Skinner & Smithers 1990). However, there is also a lack of congruence with more broadly distributed species such as yellow mongoose or springhare. The genetic variation in both these species was characterized by weak geographic structuring. While the effect of the different geographic sampling regimes over the compared studies should not be neglected, the intraspecific phylogeny of the scrub hare has probably been shaped by different evolutionary forces and historical events and may also reflect differences in dispersal capabilities, range dimensions or ecological plasticity.

The genetic gap characterizing the second phylogeographic discontinuity in the scrub hare separated a central phylogroup from a northern assemblage. The number of steps

distinguishing these two phylogroups was relatively large (more than seven mutational steps in the TCS cladogram), but the mtDNA haplogroups were not completely spatially exclusive (mixture between phylogeographic categories I & II; Avise *et al.* 1987; Avise 2000). The geographic area where haplotypes from C and N were recorded sympatrically comprised localities drawn from the northeastern parts of South Africa tracking a transition zone between the grassland and the savanna biomes in the country (Rutherford & Westfall 1994). It seemed feasible that the break between the C and the N haplogroups coincided with the border between the more mesic, elevated highveld grassland zone and the dryer lowveld savanna zone. This pattern would be consistent with the phylogeographic discontinuities between two rock hyrax lineages (Prinsloo & Robinson 1992) and between two species of red rock rabbits (Matthee 1993). That would suggest that the evolutionary history of taxonomically independent lineages of South African small mammals may have been influenced by similar vicariant events during the Pleistocene and Holocene.

A comparative evaluation across *Lepus*-species suggests that factors such as the age of the species, its effective population size, its distribution as well as the marker sets used influence the phylogeographic structuring. The detection of three distinct phylogeographic groupings within southern African scrub hares contrasts the observations of unpartitioned gene pools and panmictic networks of gene flow for brown hares (*L. europaeus*), mountain hares (*L. timidus*) and Iberian hares (*L. granatensis*; Suchentrunk *et al.* 1999; Suchentrunk & Alves 2001). The lack of phylogeographic structure in the mountain hare and the Iberian hare may be due to their relatively recent origin (middle Pleistocene), small population sizes, and restricted ranges. The scrub hare (*L. saxatilis*), in contrast, has a large population size (703 125 effective female breeders), and is distributed over the entire southern African sub-continent (it may even extend into the central and northern parts of Africa – see Flux & Angermann 1990). Moreover, the studies by Suchentrunk *et al.* (1999) and Suchentrunk & Alves (2001) on the European hare (*L. europaeus*) relied on allozymes, biparentally inherited markers that may not detect genetic partitioning resulting from female philopatry in the presence of male dispersal. That this seems likely is suggested by the high degree of population differentiation in European hares (*L. europaeus*) detected by Thulin *et al.* (1997) and

Mamuris *et al.* (2001a, b) using mitochondrial markers. Furthermore, Biju-Duval *et al.* (1991) and Perez-Suarez *et al.* (1994) reported on two well-differentiated mtDNA clades in the European rabbit and the European hare, respectively, suggesting that female philopatry is a shared trait among all these species.

Conservation units in L. saxatilis

Based on the deep branch separation of the SW assemblage and the reciprocal monophyly relative to N and C in the mitochondrial CR-I gene tree, this group renders the status of an ESU *sensu* Moritz (1994a, 1995). The microsatellite-based analyses corroborated the designation as an ESU with significantly different allele frequencies between SW and the other two populations at the six nuclear loci as indicated by significant F_{ST} and R_{ST} values as well as high accuracies of assignment tests. The fact that only two of the six loci produced significant results between SW and C or N using the Fisher's exact tests for genetic differentiation may either be an artifact due to null alleles or small sample sizes, or it may reflect an intermediate situation. Avise (2000) pointed out that it is difficult to universally define how many loci must show significant allele frequency differences for populations to warrant ESU designation. Since the contribution of the SW assemblage to the overall genetic diversity of the species was found to be substantial (see Waples 1991) and given that the mtDNA analyses have clearly demonstrated a separate, long-term evolutionary history for this population, it seems appropriate to treat it as an ESU. A logical extension from a conservation perspective will be that artificial translocation of animals between SW and the other two geographic populations should be avoided. Furthermore, the restricted distribution of the SW assemblage (it is limited to the extreme southwestern corner of South Africa) highlights the need for habitat protection and hunting regulations in the region. At least, field surveys should be carried out to assess and monitor the status of this form. The paucity of ecological data emphasizes the need for further studies on the biological distinctiveness of the taxon and the degree of ecological exchangeability *sensu* Crandall *et al.* (2000) of the SW group.

Management units (MUs) may be defined as populations that exchange only few migrants and thus are genetically distinct as well as currently demographically independent (Moritz 1994a, 1995). With regards to the mitochondrial CR-I haplotypes, the C and N scrub hare populations qualify as MUs based on the relatively small number of female migrants and the genetic distinctiveness as determined via the NCA. Microsatellite analyses also indicated significant differences in allele frequencies (measured by F_{ST} and significant Fisher's exact test results at locus Lsa 6). The high estimate of gene flow between the two populations based on the microsatellites was probably the consequence of male-biased dispersal. Since female dispersal was much lower, these two populations could still be regarded as demographically autonomous even in the absence of significant spatial structure as registered in nuclear genes (see Avise 2000). Despite the highly vagile males, females are unlikely to rapidly recolonize an area after a local population crash (Moritz 1994a), underscoring the importance of the mtDNA distinction between C and N and its implications for the management of this species.

This study has revealed that the southern African scrub hare consists of two historically isolated lineages that should be treated as separate conservation units.

Chapter 6

Population structure and history of the South African Cape Hare (*Lepus capensis*) based on mitochondrial DNA and microsatellites

6.1 Abstract

The Cape hare (*Lepus capensis*) is a small, highly mobile mammal with a widespread distribution throughout the African continent. The species is thought to be capable of dispersing over considerable distances and consequently the genetic differentiation between geographically defined populations is expected to be minimal. We determined the genetic diversity over six nuclear microsatellite loci and a 420 basepair long fragment of the mitochondrial DNA (mtDNA) control region I in twelve geographic populations of Cape hares from South Africa and Namibia. Within 53 specimens we detected 33 unique mtDNA haplotypes that defined two highly divergent phylogroups, SC and N, occupying the south-central and the northern parts of the investigated region, respectively. The time to the most recent common ancestor for all South African Cape hares was calculated as 230 940 years before present (BP) and the divergence time of the two assemblages as 66 112 years BP. Mismatch distribution analysis indicated sudden population size expansions and nested clade analysis identified contiguous range expansion as the major historical factors in the evolution of the species. Nuclear genetic differentiation between the two populations was confirmed by significant F_{ST} and R_{ST} values, as well as by high scores in assignment tests. No genetic support was retrieved for the subspecies taxonomy of *L. capensis* in South Africa, but the populations SC and N represent two evolutionarily significant units.

6.2 Introduction

The Cape hare (*Lepus capensis*) belongs to the family Leporidae in the mammalian order Lagomorpha (Walker 1964). The distribution of the species ranges over the African, European, and Asian continents (Roberts 1951; Flux & Angermann 1990), and its preferred habitat is arid areas and open grassland (Skinner & Smithers 1990). Like other hares, the Cape hare exhibits a high reproduction rate and fast population turnovers and is thought to be highly vagile (Schneider 1990).

Lagomorphs play an important role in many ecosystems as medium-sized herbivores that sustain a multitude of predators (Chapman & Flux 1990). For the healthy functioning and maintenance of these ecosystems the conservation of lagomorphs is crucial. The International Union for the Conservation of Nature (IUCN) has identified genetic diversity as one of three aspects of organismal diversity that requires conservation (McNeely *et al.* 1990). Furthermore, the IUCN lagomorph specialist group recognized the clarification of the still confused taxonomy and systematics within the genus *Lepus* as a prerequisite for adequate conservation measures and called for molecular studies towards this aim (Chapman & Flux 1990). Moritz (1994 a, b; 1995) provided a genetic framework for the identification of conservation units within species with his definition of the concepts of evolutionarily significant units (ESUs) and management units (MUs). The detection of genetically defined ESUs and/or MUs in the South African Cape hare may be of special relevance, since the subspecies taxonomy for this species on the subcontinent has been based solely on fur coloration and geographic locality (Roberts 1951). In South African small mammals, local differences in fur color are often observed and are thought to be a consequence of climate and habitat rather than genotype (Roberts 1951). It has been criticized to base subspecies delineations on traits with uncertain genetic underpinnings (Avise *et al.* 1987) or on morphological characters that exhibit phenotypic plasticity (Zink & Avise 1990), since these traits tend to reveal ecological associations rather than evolutionary relationships. A critical, conservation-oriented re-evaluation of the subspecies designations within *L. capensis* should therefore include information on genetically defined intraspecific units and their phylogenetic relationships.

Based on fossil evidence, the genus *Lepus* is thought to have appeared on the African continent in the late Pliocene or early Pleistocene some 4-1.5 million years ago (Lavocat 1978; Carroll 1988). During this period, the climate in Africa experienced cycles of cold and dry phases alternating with warm and humid phases (Grubb 1978; De Menocal 1995). The range of savanna habitats extended and that of humid forests contracted in the dry episodes, and the reverse occurred during moist periods (Coe & Skinner 1993). The evolution of African mammals has been influenced profoundly by these Pleistocenic oscillating habitat expansions and contractions, which are thought to have induced diversification events at the species and subspecies level (Ewer & Cooke 1964; Grant & Leslie 1993). The geographic partitioning of the genetic variation in species may often reflect the legacy of these past climatic events and thus allows the reconstruction of evolutionary histories. In chapters 4 and 5 we found signals of population diversification and population size expansions reflected in the mtDNA variation of the southern African scrub hare, *L. saxatilis*. These events, as well as the coalescence time to the most recent common ancestor for all scrub hares, were dated back to the Pleistocene. Moreover, based on mtDNA and microsatellites three phylogeographic assemblages were identified in the southern African scrub hare: a southwestern, a central, and a northern lineage. Phylogeographic hypotheses gain credence when similar historical and geographic patterns are discovered for several species (Grubb 1978; Avise 2000). It was therefore considered to be of interest to compare the population history and phylogeography of the scrub hare to that of the other South African hare species, the Cape hare, since their biology and demography are very similar.

In this study we investigated the amount and the geographical structuring of genetic variation of the South African Cape hare using rapidly evolving CR-I sequences and microsatellites. The main objectives were (i) to assess whether the genetic variation in South African *L. capensis* is geographically structured, (ii) to elucidate the evolutionary history of the Cape hare in this region, (iii) to determine if any of the included Cape hare subspecies represent distinct evolutionary lineages, and (iv) to compare the phylogeographic pattern of *L. capensis* to that of other South African small mammals, specifically the scrub hare.

6.3 Materials and methods

Samples and DNA extraction

Tissue samples from 67 Cape hares were obtained in 1999 and 2000. Four animals were collected in Namibia, with the balance (63 hares) sampled from 11 different localities throughout South Africa (Fig. 6.1). Fresh muscle and heart tissue was transferred into a 20% DMSO/saturated salt solution (Amos and Hoelzel 1991) and stored at room temperature. Total genomic DNA was prepared by the proteinase K-phenol-chloroform extraction followed by ethanol precipitation (Sambrook *et al.* 1989).

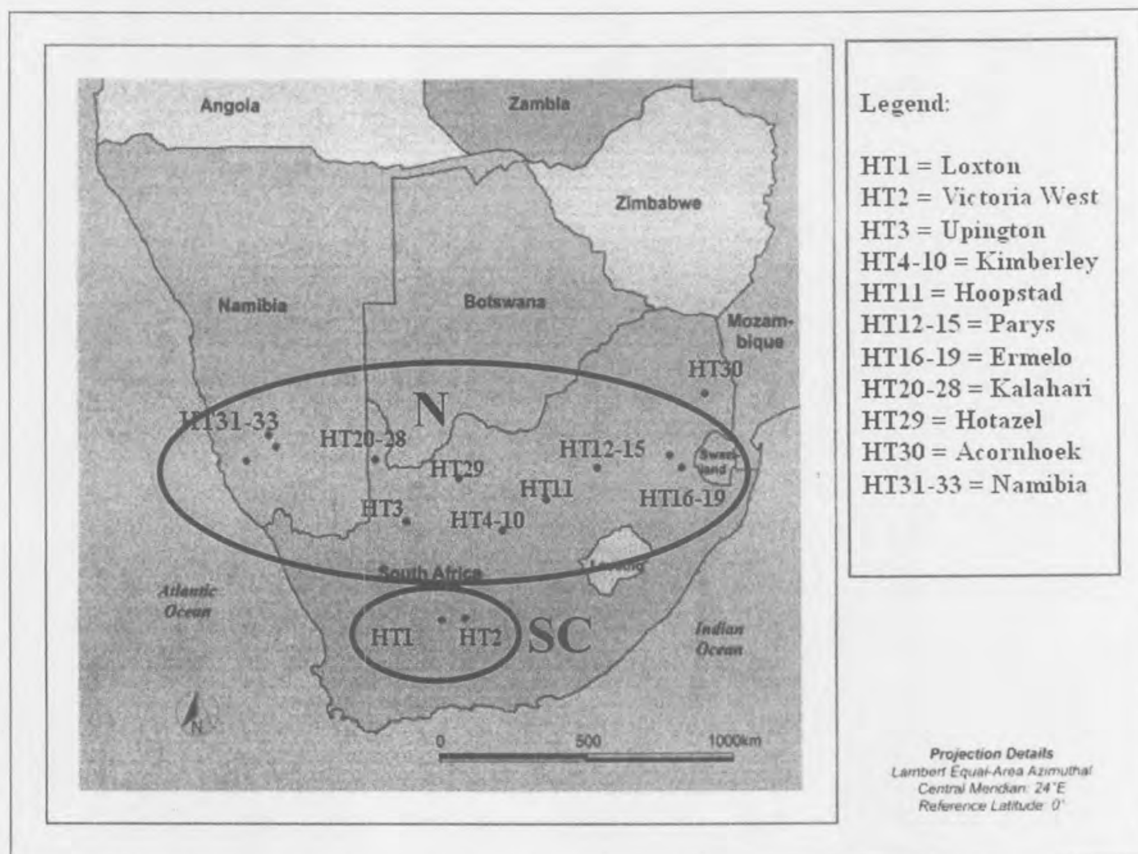


Fig. 6.1 Collection localities and corresponding mtDNA haplotypes of *Lepus capensis*. See Table 6.1 for subspecies designations and number of individuals per haplotype. The encircled areas indicate the two phylogeographic assemblages identified via NJ and MP analysis (SC = south-central and N = north; see Results).

Data collection mtDNA

Control region amplification and sequencing. The non-coding control region (CR) is recognized for its utility in investigating intraspecific relationships (Harrison 1989; Simon *et al.* 1994; Baker & Marshall 1997). Given the wide utility of the hypervariable 5' end of the mitochondrial control region (CR-I) as a population marker (O'Corry-Crowe *et al.* 1997; Garcia-Rodriguez *et al.* 1998; Stepien & Faber 1998; Houlden *et al.* 1999; Pierpaoli *et al.* 1999; Tarr & Fleischer 1999; Pope *et al.* 2000; Davison *et al.* 2001; Girman *et al.* 2001; Grau Nersting & Arctander 2001; Jensen-Seaman & Kidd 2001; and Sivasundar *et al.* 2001 among others), we selected this region for the present study. A 420 bp long fragment of the 5' end of the CR-I of 53 Cape hares was amplified by polymerase chain reaction (PCR), purified, cycle sequenced, and electrophoresed following Kryger *et al.* (submitted; chapter 4).

Data collection microsatellites

Six microsatellite loci (Lsa1, 2, 3, 4, 6 & 8) originally isolated and characterized for the scrub hare (*L. saxatilis*; Kryger *et al.* in press; chapter 2) were scored for 68 Cape hares (see chapter 3). Over and above the 53 animals screened for mtDNA variation (Table 6.1), we genotyped eight additional specimens from Victoria West, three collected in the Kalahari, two obtained from Parys, and one specimen each from Loxton and Upington. PCR reactions were performed as described in Kryger *et al.* (in press; chapter 2). Products were run on an ABI 377 automated sequencer and scored against GENESCAN-500 Tamra (PE, Applied Biosystems) as the internal size standard using the GENESCAN and GENOTYPER software (PE, Applied Biosystems).

Data analysis mtDNA

Alignment. The corresponding L- and H-primer sequences of each individual were aligned, compared and edited in SEQUENCE NAVIGATOR (PE, Applied Biosystems). The

consensus sequences were subsequently automatically aligned using CLUSTAL X (Thompson *et al.* 1997) and manually optimized in order to eliminate excess gaps. The sequences were deposited in GenBank (Accession nos AF491353 - AF491385).

Phylogenetic reconstruction. Analyses to retrieve phylogenetic associations among lineages were performed in PAUP*4.0 beta 10 version (Swofford 2002). We rooted the phylogenetic trees against *L. europeaus,* the European hare, since this species is thought to be a close relative of the Cape hare (Angermann 1983; Suchentrunk *et al.* 1998a,b). We ran a maximum likelihood ratio test as implemented in MODELTEST Version 3.04 (Posada & Crandall 1998) in order to determine the model of nucleotide substitution that best fits our data. The HKY85 model (Hasegawa *et al.* 1985) with gamma correction (Gu & Zhang 1997) obtained the highest likelihood score (with a statistical significance of $P = 0.01$) and was thus selected for the neighbor-joining (NJ) and the maximum likelihood (ML) analyses. The Ti/Tv ratio as well as the shape parameter (α) for the gamma distribution from the presented dataset were estimated using the maximum likelihood method in PAUP. A gene tree containing all the haplotypes was constructed with the neighbor-joining algorithm (Saitou & Nei 1987) based on the pairwise distances and using the option of randomized tie breaking. For the maximum parsimony (MP) analysis (Hennig 1966; Felsenstein 1982, 1988), input order was randomized and the alignment gaps were treated as a fifth character state and coded following Simmons & Ochoterena (2000) in order to account for the insertions/deletions present among members of the ingroup taxa. Nodal support for the trees was assessed by 1000 (NJ and MP) bootstrap iterations (Felsenstein 1985).

Polymorphism and partitioning of genetic variation. The hierarchical components of the mtDNA variation were computed under the AMOVA framework (Weir & Cockerham 1984; Excoffier *et al.* 1992) as explained in chapter 4. We tested two different hypotheses: (i) each group corresponds to a subspecies as described by Roberts (1951); and (ii) the groups corresponds to the clades as derived by the phylogenetic MP analysis. An exact test for population differentiation, interpopulational divergence time, pairwise intrapopulational difference ($\pi_X = D_X$), the average interpopulational difference

(π_{XY} = Nei's raw D_{XY}), the corrected average interpopulational difference (($\pi_X + \pi_Y$)/2 = Nei's net D_A), haplotype diversity (H), average number of pairwise nucleotide differences (k, Tajima 1983), and the nucleotide diversity (π) were calculated as in chapter 4. We tested for the assumption of neutrality of the analyzed CR-I sequences by computing Fu and Li's D*, Fu and Li's F* , and Tajima's D statistics (Fu & Li 1993; Tajima 1989a) as implemented in DNASP.

Mutation rate and coalescence. We calculated the mutation rate and the coalescence time (= time to the most recent common ancestor, TMRCA) of the CR-I haplotypes of the Cape hare following Rooney *et al.* (2001; see chapter 4 for details).

Mismatch distributions. Mismatch distribution analyses were performed under the sudden expansion model proposed by Rogers & Harpending (1992) as implemented in ARLEQUIN, and θ_0, and θ_1 were estimated in three different ways as outlined in Kryger *et al.* (submitted; chapter 4).

Nested clade analysis. Since TCS did not accept the input file with the *L. capensis* CR-I sequences, it was not possible to estimate a 95% parsimony cladogram for our data set. We therefore used an unrooted NJ network constructed in PAUP to determine the nested clade structure for our data set following the nesting rules described in Templeton *et al.* (1987) and Templeton & Sing (1993). A nested clade analysis applying the methods described in Templeton *et al.* (1995) was performed using the program GEODIS (Posada *et al.* 2000) with the aim of separating historical from contemporary processes. The results of the NCA were interpreted according to Templeton *et al.* (1995), Crandall & Templeton (1996), and the new NCA key inference (2001) downloaded from the NCA webpage (http://zoology.byu.edu/crandall_lab/geodis.htm).

Data analysis microsatellites

Hardy-Weinberg equilibrium (HWE) and genotypic disequilibrium. To test for selective neutrality, we computed the expected genotypic frequencies under random mating and

likelihood ratio (G^2) tests for HWE at each locus. The quantification of genetic diversity in each population was obtained via the calculation of observed and expected heterozygosities (H_O and H_E) at each locus, the mean H_O and H_E for each population, and the total number of alleles at each locus and over all loci. These parameters were retrieved with POPGENE Version 1.31 (Yeh *et al.* 1997). We used Fisher's exact tests as implemented in GENEPOP Version 3.2a (Raymond & Rousset 1995a) to test for linkage disequilibrium between loci (see chapter 5 for details of these procedures).

Phylogenetic reconstruction. The program POPULATIONS Version 1.2.26 (Langella 1999-2002) was applied to compute Nei's genetic distance, D_A (Nei *et al.* 1983), for all pairwise comparisons among sample locations, to construct a neighbor-joining tree based on these distances (Takezaki & Nei 1996), and to calculate 1000 bootstrap iterations for the nodes in the NJ tree (for details see chapter 5). The tree was displayed and saved as a graphic file in the computer application TREEVIEW (Page 1996).

Population structure. In order to test for population structuring of the genetic variation, we performed Fisher's exact tests for population differentiation (Raymond & Rousset 1995b; Rousset & Raymond 1997). Furthermore, we estimated Wright's fixation index, F_{ST} (Wright 1951, 1965; Weir and Cockerham 1984), and the unbiased multilocus R_{ST} statistics (Slatkin 1995) between the phylogenetic lineages. Pairwise R_{ST} statistics were also computed among all subspecies represented with a large enough sample size. Additionally, assignment tests were run as implemented in the program GENECLASS (Cornuet *et al.* 1999; for details see chapter 5).

Migration rates. To test for the amount of gene flow, coalescence theory derived maximum likelihood estimates for the migration rates (Beerli & Felsenstein 2001) among the different populations were calculated based on the microsatellite data using a Markov chain Monte Carlo approach as implemented in MIGRATE Version 1.1 (Beerli 1997-2001) as described in chapter 4.

6.4 Results

Mitochondrial DNA

Cape hare mtDNA CR-I. The electropherograms were clear and showed no ambiguous peaks (and therefore no heteroplasmy) throughout the sequenced product. The 420 bp fragment of mitochondrial CR-I included in the analyses comprised 116 variable sites (27.62%), of which 88 (20.95%) were parsimony informative, one was an indel (position 265). The Ti/Tv ratio was relatively high (12.27), but well within the range of values for other vertebrate CRs. Kryger *et al.* (submitted; chapter 4) and Pierpaoli *et al.* (1999) reported on CR-I Ti/Tv ratios of 11 in the South African scrub hare and 3.9 among hare species from Europe, respectively. The high Ti/Tv ratio, together with the high intraspecific variation (see below), conformed closely to the expected characteristics of mtDNA CRs. We therefore considered it highly unlikely that we had amplified any nuclear paralogues (Lopez *et al.* 1994; Zhang & Hewitt 1996). Heterogeneity in substitution rates among nucleotide sites was observed (α = 0.7134). The nucleotide composition was characterized by a scarcity of G (only 9% versus approximately 30% for each of the other nucleotides), which is typical for vertebrate CRs (Baker & Marshall 1997).

CR-I haplotypes and polymorphism. The sequenced CR-I fragment defined 33 unique mitochondrial haplotypes among the 53 sample specimens (Tables 6.1 and 6.2; for complete alignment see Appendix Table A2) resulting in a high haplotype diversity estimate for the South African population of the species (H = 0.97; Table 6.3). Most sample localities were characterized by private haplotypes (except Hoopstad and Parys that shared HT11); 22 haplotypes were singletons (Table 6.1).

The MP analysis found 2 shortest trees with a length of 260 steps that had a moderate consistency index and a relatively high retention index (CI = 0.423, RI = 0.708), indicating a moderate level of homoplasy in the dataset. The ratio of parsimony informative characters (88) to the number of OTUs (33) was high for an intraspecific dataset and explains the high bootstrap support values retrieved for the major clades in

the parsimony analysis which were identical with the two clusters retrieved in the NJ analysis (Fig. 6.2). The phylogenetic tree clearly revealed two major assemblages (Fig. 6.2). The first assemblage (SC) consisted exclusively of animals originating from the two most southern sample localities (Loxton and Victoria West; Fig. 6.1) and was highly supported (bootstrap value 95%). This clade comprised only four animals with two haplotypes. The second assemblage (N) united animals from all other sample localities except Acornhoek and was also supported by high bootstrap values (93-96%). This cluster was characterized by a star-shaped topology with short internal branches (Fig. 6.2) and the haplotypes did not group according to their geographic origin or subspecies category (Table 6.1). The two groups were treated as populations in the further analyses. The sequence divergence between the two haplotypes in cluster SC was high (9.95%). Sequence divergences ranged from 0.25-14% among the haplotypes of group N and from 13.41-25.51% between the two populations (Table 6.3; for all pairwise sequence divergences see Appendix Table A4). Specimen X01 from Acornhoek had a highly divergent haplotype (HT30; 13.57-18.12% sequence divergence to any other HT) and clustered outside the two major clades (Fig. 6.2). The divergences against the outgroup sequence (*L. europaeus*) were approximately 39% for the SC haplotypes, 36.48% for the haplotype from Acornhoek, and ranged from 31.75-47.06% for the northern group haplotypes (Table A4 in Appendix). Within assemblage haplotype diversity was moderate in SC (0.667) and very high in N (0.974). The average number of nucleotide differences was approximately 20 in both clusters, and nucleotide diversity was 0.049 in both sub-populations (Table 6.3).

Table 6.1 Sampling locations, haplotype designations (HT), and field numbers of the animals included in this study. Note that the haplotype numbering corresponds approximately to the geographical origin of the specimens, starting from the southwestern Cape.

Assemblage	Subspecies	Population	HT	Field number of animals with this haplotype
SC	*L. c. centralis*	**1 – Loxton**	1	CL1, CL2
			only microsatellites	CL3
SC	*L. c. centralis*	**2 – Victoria West**	2	CV1, CV2
			only microsatellites	CV3-CV10
N	*L. c. kalaharicus*	**3 – Upington**	3	I01
			only microsatellites	I02
N	*L. c. vernayi*	**4 - Kimberley**	4	J01
			5	J02
			6	J03, J05
			7	J04
			8	J08, J09
			9	CK1
			10	CK2
N	*L. c. hartensis*	**5 - Hoopstad**	11	M01, M02, M03, O05
N	*L. c. ochropus*	**6 – Potchefstrom**	12	N01
N	*L. c. ochropus*	**7 - Parys**	13	O01, O03, O06
			14	O02
			15	O04, O07, O08
			only microsatellites	O09 & O10
N	*L. c. ermeloensis*	**8 – Ermelo**	16	P01, P02, P08, P10, P11, P12
			17	P03
			18	P05, P06, P07
			19	P09
N	*L. c. kalaharicus*	**9 - Kalahari**	20	Q01
			21	Q02
			22	Q03
			23	Q04
			24	Q05
			25	Q06
			26	Q07
			27	Q08
			28	Q10
			only microsatellites	Q11-13
N	*L. c. hartensis*	**10 - Hotazel**	29	R01
N?	*L. c. bedfordi*	**11 - Acornhoek**	30	X01
N	*L. c. mandatus*	**12 - Namibia**	31	Z05
			32	Z08
			33	Z09

Table 6.2 Variable sites defining 33 CR-I haplotypes for 53 *Lepus capensis* specimens. For geographic localities and the number of animals carrying a specific haplotype see Fig. 6.1 and Table 6.1.

	1111111111111111111111111111111222222222222222222222222222222222222222222223333333333333333333333333
	111222445557888999000111222334445556666668999990111122222222333344444455556667777777899990000111222344556666777777
	812436746126601323802923478956178089012347202367912789012346890379014567135604501345685123901591240789040223490134573
HT1	ACAACTGCCAGTCTACTTTTATCCCAATCAAACCTTGTCCTCTCTGTCTTACTTACCACTCATCTTTGACCACTTTTG-CTCCCCTAACACATCAGCATCTATCTTTTGGAGCTAT
HT2	..........A.T..A.........C..TG....C....T..C...C..C.T.......C..CT.CCA....T.C.C.......C............CT.C.TCCC..A......
HT3	G..GTCA.T..CT.......T.TT.T...G.........T.ACT.AATAA....GT..TCTC.TGC.A......G.CA....T....C....CTGA...T.T......A..A....
HT4	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..TATAA....GT.....CCTG..A..T..CA..........C.T....CTGA...T.T....C.AA.AT...
HT5	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..AATAA....GT.....CCTG..A..T..CA..........C.T....CTGA...TCT....C.A..AT...
HT6	G..GTCA.T..CT......CT.TT.T..TG.........T.ACT.AATAA....GT..TC.CCTGC.A.TT...G.CA.......C.T.....TGA...TCT.....CA..A....
HT7	G..GTC..TG.CT.....C.T..T.T..TGGTTTC.A..TCAC..AA.AC.....T.....CCTG.C.......G..A.........T...G.......TCT.....CA..A.CG.
HT8	G..GTCA.T..CT......CT.TT.T.CTG......A..T.AC..TATAA....GT.....CCTG..A..T..CA....T.....C.T....CTGA...T.T....CCAA.A....
HT9	...GTC..T..CT.....CCT..T.T...G.......CTTCAC...ATAAG...G.T.TC.TCTGCCA.T...CG.CA.......C.T....CTGA...TCT......AA.AT...
HT10	G..GTC..T..CT......CC.TT.T...G......A....AC..AATAA....GTT..C.CCTG.CAG.T..CC.CA..C....CTT.G.....A...T.T......AAG.....
HT11	G..GTC..T..CT.....CCC.TT.T.....GT......TCAC..AATAA....GT..TC.CCT..CA.T...CG.CA.......C.T....CTGA...TCT......AA.AT...
HT12	G..GTC..T..CT..T....T.TT.T..TG......A....AC..AATAA....GT.....CCTG..A..T..CA..AA....T.C.TT.T.CTG....T.T......A..A....
HT13	G..GTCA.T..CT.......T.TT.T..TG.........T.AC..AATAA....GT...C.CCTGC.A.TT...G.CA.........T.....TGAT..TCT.....CA..AT...
HT14	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..TATAA....GT.....CCTG..A..T..CA....T.....C.T....CTGA...T.T....C.AA.AT...
HT15	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..TATAA....GT.....CCTG..A..TG.CA....T.....C.T....CTGA...T.T....C.AA.AT...
HT16	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..AATAA....GT.....TCTG..A..T..CA..........C.T....CTG....TCT....C.A.GAT...
HT17	G..GTC..TG.CT.....C.T..TTT...G.C..CCA..T.AC..AA.AC....GTTC.C.CCTG.CA......G.CA..C....C.T...........TCT.....CAA.A.CG.
HT18	G..GTCA.T..CT......CT.TT.T..TG......A..T.AC..AATAA....GT.....TCTG..A..T..CA..........C.T....CTG....TCT....C.A..AT...
HT19	G..GTC..TG.CT.....C.C..T.T..TG.C..CCA..T.AC..AA.AC....GT...C.CCTG.CA......GCCA.......C.T...G.......TCT.....CA..A.CG.
HT20	G..GTC..T.ACT......CT.TT.T..TG......A..T.AC..AATAA.....T...C.TCTG..A.TT...G.CA......TC.T....C.GA...T.T......AA..T.GC
HT21	G..GTCA.T.ACT......CT.TT.T..TG.........T.ACT.AATAA....GT...C.CCTGC.A.T...CG.CA.........T.....TGA...TCT.....CA..AT...
HT22	G..GTCA.T..CT.......T.TTTT...G.........T.AC..AATAA....GT...C.CCTGC.A.T....G.CA.......C.T....CTG....T.T......A..AT...
HT23	G..GTCA.T..CT.......T.TT.T...G.........T.AC..AATAA....GT...C.CCTGC.A.T....G.CA.......C.T...GCTG....T.T......A..AT...
HT24	G..GTCA.T.ACT......CT.TT.T..TG.........T.ACT.AATAA....GT..TC.CCTGCCA.T..ACG.CA.......C.T.....TGA...TCT.....CA..AT...
HT25	G..GTC..T..CT......CT..T.T..TG.GT......TCAC..AA.AAG...GT..TC.CCTGCCA.T...CG..A......T..T.....TGA...TCT......AA.AT...
HT26	GT..TC..T..CT......CTCTT.T...G......A..TCA.TC.ATAA....GT...C.CCTAC.A.T...CG.CA.......C.T....CTGA...T.T......A..A.C..
HT27	G.G.TC..T..CT......CTCTT.T..TG..T...A..T.ACT.AATAA..C.GT...C.CCTG..A.TT..CG.CA.........T.....TGAT..T.T......A.GA.C..
HT28	G..GTC..T..CT......CTCTT.T..TG..T...A..T.ACT.AATAA.....T...C.CCTG..A.TT..CA.CA.........T.....TGATG.T.TC.....A...TC..
HT29	G..GTC..T..CT.......T.TT.T..TG......A..T.AC..A.TAA.....T.....C.TG..A..TG..A..A.......C.T....CTG....T.T......A..A....
HT30	...G...TT....AT.CC.CT.TTAT...G.....CT..TCTC..AAT.C...CT...TC..CT.ACA...T..C....T....T..T......G....T.T..C...A.......
HT31	G..GTC..T..CT......CTCTT.T..TG..T...A..T.ACT.AATAA....GT...C.CCTGC.A.TT..CG.CA.......C.T.....TGAT..T.T......A..ATC..
HT32	G..GTC..T..CT.....CCCC.TTTG.TG.GT......TCACT.AATAA..C.G....C.CCTGCCA.T...CG.CACTCAT..C.T....CTG....T.T......A..AT...
HT33	...GTC..T..CT.....CCTCTT.T..TG..T..C...TCACT.AATAG....GT...C.CCTGCCA.T...CG.CA..C....C.T.....TG....TCT......AA..T...

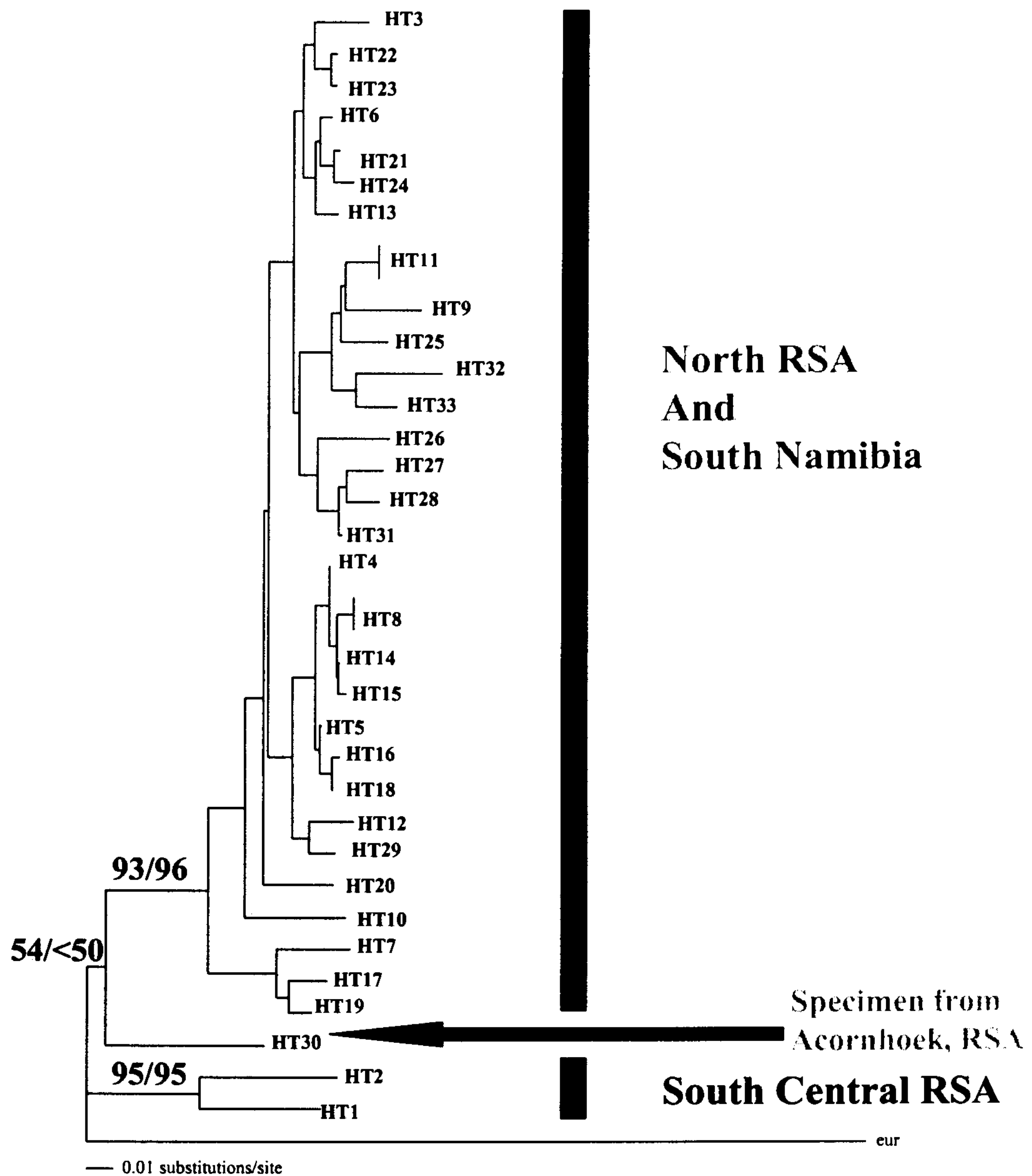


Fig. 6.2 NJ phylogram for *Lepus capensis* based on 420 bp of mtDNA CR-I sequence, HKY85 Γ-corrected and rooted against *L. europaeus* (= eur). For the designations and geographical origins of the 33 haplotypes see Table 6.1 and Figure 6.1. The MP analysis produced the same topology. Numbers at nodes refer to the NJ/MP bootstrap support after 1000 replicates; bars on the right indicate the phylogeographic assemblages.

Table 6.3 MtDNA CR-I diversity estimates in two phylogeographic groups of the Cape hare (SC = south-central and N = northern); n = sample size, s = polymorphic sites, k = average number of nucleotide differences, no. of HT = number of CR-I lineages, H = haplotype diversity and π = nucleotide diversity (standard deviations are in parenthesis), pairwise sequence divergences estimated under the HKY85 model with γ-correction.

Assemblage	**n**	**s**	**Ti/Tv**	**k**	**no. of HT**	**H**	π	**% Paiwise divergence**
SC	4	30	27/3	20.00	2	0.667 (0.204)	0.049 (0.015)	9.95
N	49	93	87/10	19.93	31	0.971 (0.011)	0.049 (0.004)	0.25 - 14
Total	53	116	107/18	24.10	33	0.974 (0.010)	0.059 (0.005)	0.25 – 25.51

Mutation rate and coalescence time. We obtained a value for the average number of nucleotide substitutions per site of $d = 1.23$. Based on an interspecific divergence time estimate between the scrub hare and the Cape hare of 3.45 million years ago (Ma; see chapter 4 for details), we estimated the rate of nucleotide substitutions per site per lineage per year to be $\lambda = 1.78 \times 10^{-7}$. Given a generation time of two years in hares (Marboutin & Peroux 1995), the mutation rate per nucleotide site per generation (μ) was calculated at $2 \times 1.78 \times 10^{-7} = 3.56 \times 10^{-7}$. The mutation rate per generation per haplotype of a length of 420 bp was consequently $\nu = 1.495 \times 10^{-4}$. The coalescence time in generations was determined to be 115 470, this corresponds to a time of common ancestry of approximately 230 940 years for southern African Cape hares.

Partitioning of genetic variation. The results of an AMOVA where the groups were categorized according to Robert's (1951) subspecies designations (data not shown) yielded a significant but low Φ_{ST} value of 0.48363 and a very low Φ_{CT} of 0.083, which was not significant ($P = 0.119$). The low contribution (8.35%) of the among group variance towards the total variance underscored the absence of differentiation among the defined groups.

In contrast, the AMOVA indicated a high degree of partitioning when the groups consisted of the two assemblages defined by the NJ/MP analysis with a highly significant ($P < 0.0001$) Φ_{ST} value of 0.73 (Table 6.4). Under this scenario the largest portion of the

total variance (61.18%) was attributable to variation among groups (= assemblages) and the Φ_{CT} value of 0.61 was highly significant.

The finding of a distinct genetic differentiation between the SC and the N assemblage was further corroborated by the significant ($P < 0.05$) result of the exact test for population differentiation. In addition, Nei's raw D_{XY} and Nei's net D_A (Table 6.5) were highly significant, and the interpopulational variation clearly exceeded the already high intrapopulational variability (as indicated by Nei's D_X; Table 6.5). By solving the equation $t = \tau/2v$ with the τ-value of 19.77 (estimated as the population divergence time indicator in ARLEQUIN) we calculated the split between the two Cape hare clades at ± 132 240 years ago. All neutrality test statistics for the northern population as well as for the entire Cape hare population in South Africa were non-significant, negative values (Table 6.5) indicating the selective neutrality of the investigated sequences. In contrast, all three statistics resulted in a significant, positive value for the SC group of Cape hares which was probably an artifact resulting from the small sample size for this population.

Table 6.4 Hierarchical analysis of molecular variance (AMOVA) of CR-I sequences among the two Cape hare populations SC and N.

Hierarchy	d. f.	% Total variance	Φ Statistic		P - value
Among groups	1	61.18	Φ_{CT}	0.612	< 0.0001
Among populations	9	12.03	Φ_{SC}	0.310	< 0.0001
Within populations	41	26.79	Φ_{ST}	0.732	< 0.0001

Table 6.5 Average pairwise genetic differences (Nei & Li 1979) between two Cape hare populations (SC = south-central and N = northern): the values shown are Nei's raw D_{XY} (upper diagonal), Nei's net D_A (lower diagonal), and intrapopulational Nei's D_X as diagonal elements in bold (paiwise comparisons at a significance level of $P < 0.0001$). Results of neutrality tests all non-significant, except for SC where * = $P < 0.05$ and ** = $P < 0.02$.

	SC	N	Tajima's D	Fu & Li's D*	Fu & Li's F*
SC	**20.000**	50.469	2.29727 (*)	2.29727 (**)	2.41225 (**)
N	30.891	**19.155**	-0.83738	-1.71693	-1.66011
Total	-	-	-0.71815	-0.51310	-0.70732

Mismatch distributions. The distribution of the pairwise nucleotide differences in the entire Cape hare population (Fig. 6.3), as well as in the northern population (Fig. 6.4), was unimodal, and the goodness-of-fit statistics (sum of squared deviation and Harpending's raggedness index) were non-significant in both cases. Thus the null hypothesis of a sudden population size expansion could not be rejected. The measure for the effective maternal population sizes, the θ-values (Table 6.6), confirmed this scenario with a consistently strong increase from the initial population sizes (θ_0) to the final population sizes (θ_1). The values for the SC groups did not indicate a population size expansion, but due to the very small sample size of only four sequences these results cannot be considered reliable. However, the mismatch distribution of the entire sample showed a second small peak at 52 pairwise differences (Fig. 6.3), which may be attributable to the differentiation of the SC haplotypes.

The effective maternal population sizes (N_e) were calculated by solving the formula $\theta = 2 N_e \mu$ and using the θ-values obtained in FLUCTUATE (for reasoning see discussion in chapter 4). The approximate dates of the expansions were calculated from the τ -values obtained in ARLEQUIN (Table 6.6) applying the formula $t = \tau/2\nu$. The results of these calculations indicated that the entire South African Cape hare population increased from an initial 81 461 effective female breeders approximately 115 251 years ago to the 372 191 of present. The northern clade experienced an increase of effective female population size from an initial 70 225 to a final 433 989 animals at roughly 123 612 years ago.

Table 6.6 The θ_0 , θ_1 , and τ-values for two Cape hare populations (SC = south-central and N = northern) and for the total population estimated via three different approaches.

	Watterson/DNASP		MHMC/FLUCTUATE		N-L L-S/ARLEQUIN		
	θ_0	θ_1	θ_0	θ_1	θ_0	θ_1	τ
SC	14.832	16.364	0.025	0.006	2.761	2.763	16.25
N	8.298	23.325	0.050	0.309	4.088	73.496	18.48
Total	12.590	26.443	0.058	0.265	7.293	76.016	17.23

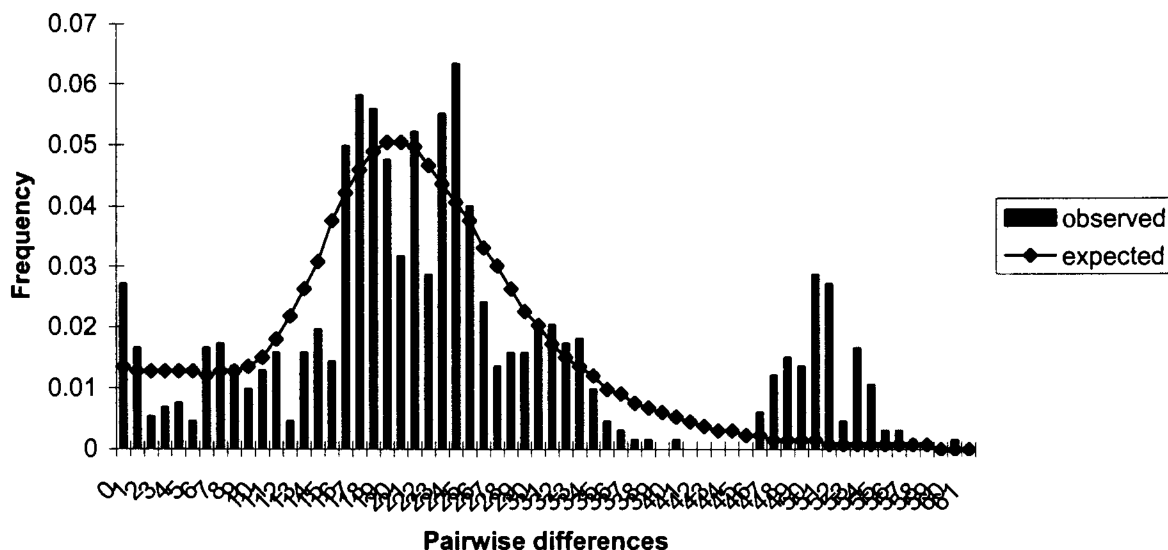


Fig. 6.3 Mismatch frequency distributions of the pairwise nucleotide differences in the total population of Cape hares, sum of squared deviation = 0.006 (P = 0.333) and Harpending's raggedness index = 0.006 (P = 0.235).

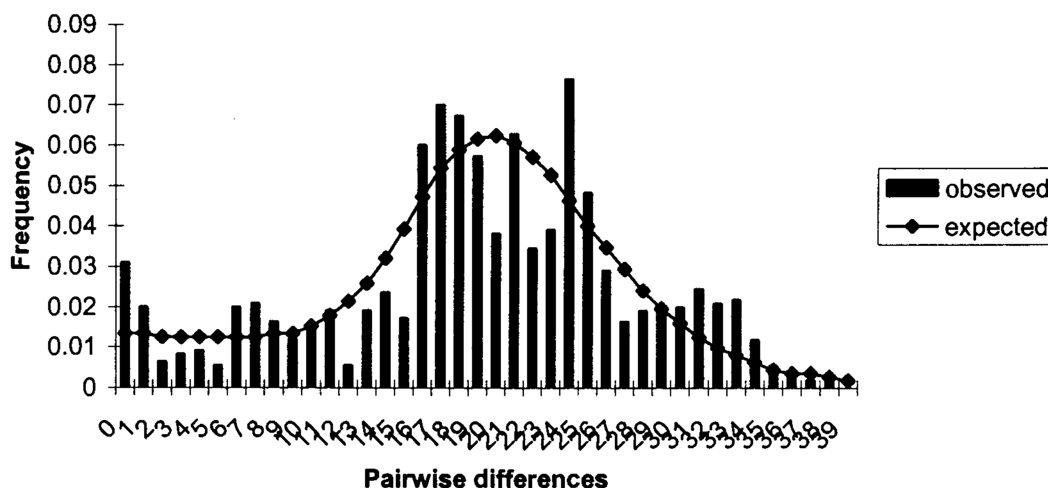


Fig. 6.4 Mismatch frequency distributions of the pairwise nucleotide differences in the northern population of Cape hares, sum of squared deviation = 0.005 (P = 0.402) and Harpending's raggedness index = 0.007 (P = 0.226).

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Haplotype network and nested design. The nested design for the CR-I haplotypes found in *L. capensis* is depicted in Figure 5. Despite the large number of mutational steps separating HT1 from HT2, they are nested in the same "one-step clade", since they consistently resolved as sister taxa in all PAUP based analyses. For the justification of collapsing longer branches and treating them as artificial "one-steps" see Durand *et al.* (1999) and chapter 5.

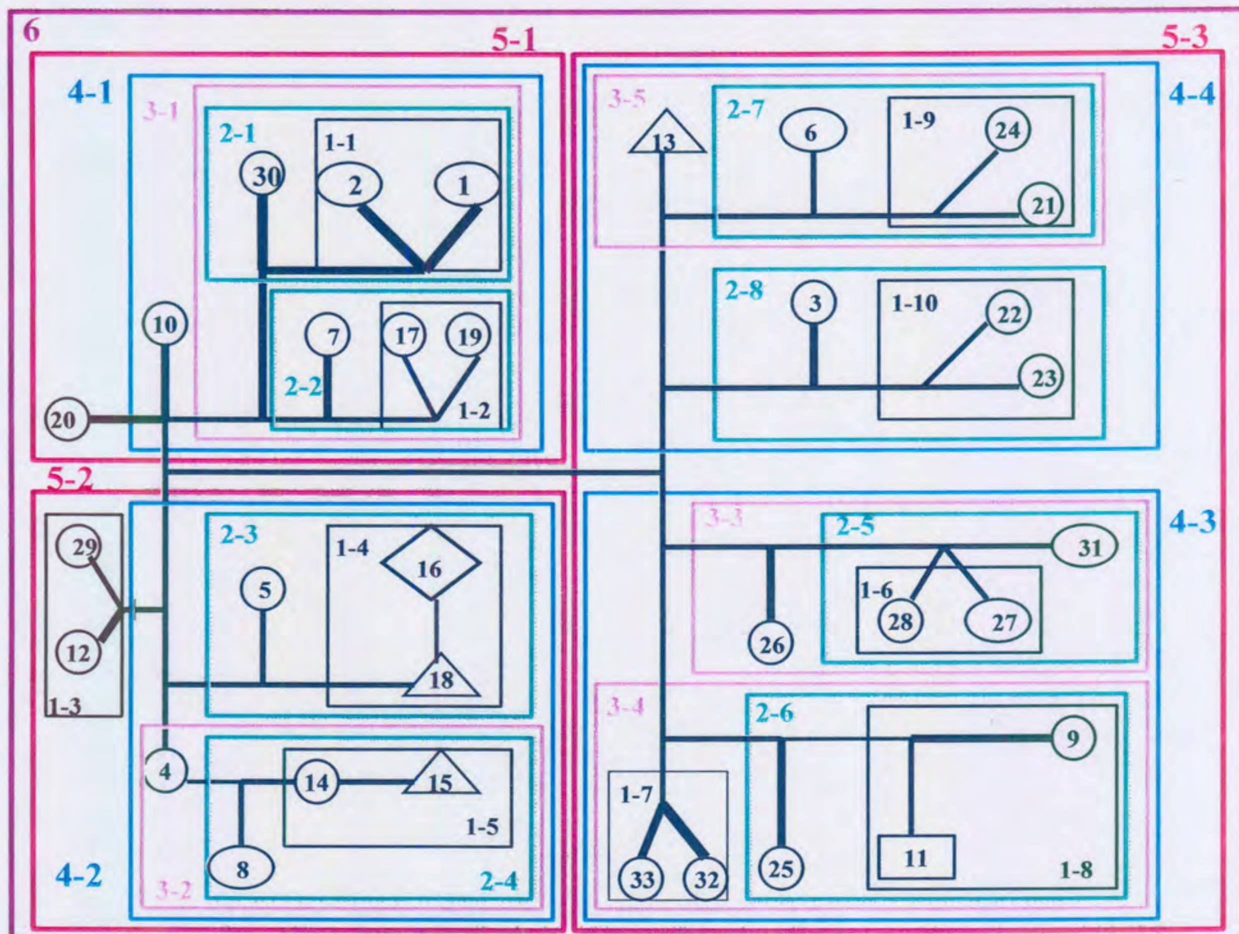


Fig. 6.5 Nested network for the mitochondrial haplotypes of *Lepus capensis*. Haplotypes are indicated by the letter code used in Fig. 6.1 and Table 6.1. Haplotypes in circles represent singletons, haplotypes in ovals and triangles occurred in two and three animals, respectively. Haplotype 11 was found four times (as symbolized by the square shape) in two different locations. Haplotype 16 was present in six animals. The thickness of the connecting lines indicates the number of mutational steps that interconnect two haplotypes: very thin lines represent connections of one mutational step, thin lines less than five steps, thick lines less than ten steps and very thick lines more than ten steps. Clade 1-1 corresponds to the SC assemblage in Fig. 6.2. HT 30 stems from the animal collected in Acornhoek, all other haplotypes and clades are representative of the N assemblage in Fig. 6.2.

Permutation analysis of the nested clades. The contingency analysis revealed four cases of significant associations of clades and sampling locations; all cases were at higher clade levels (levels 3, 4, 5, and 6; Table 6.7).

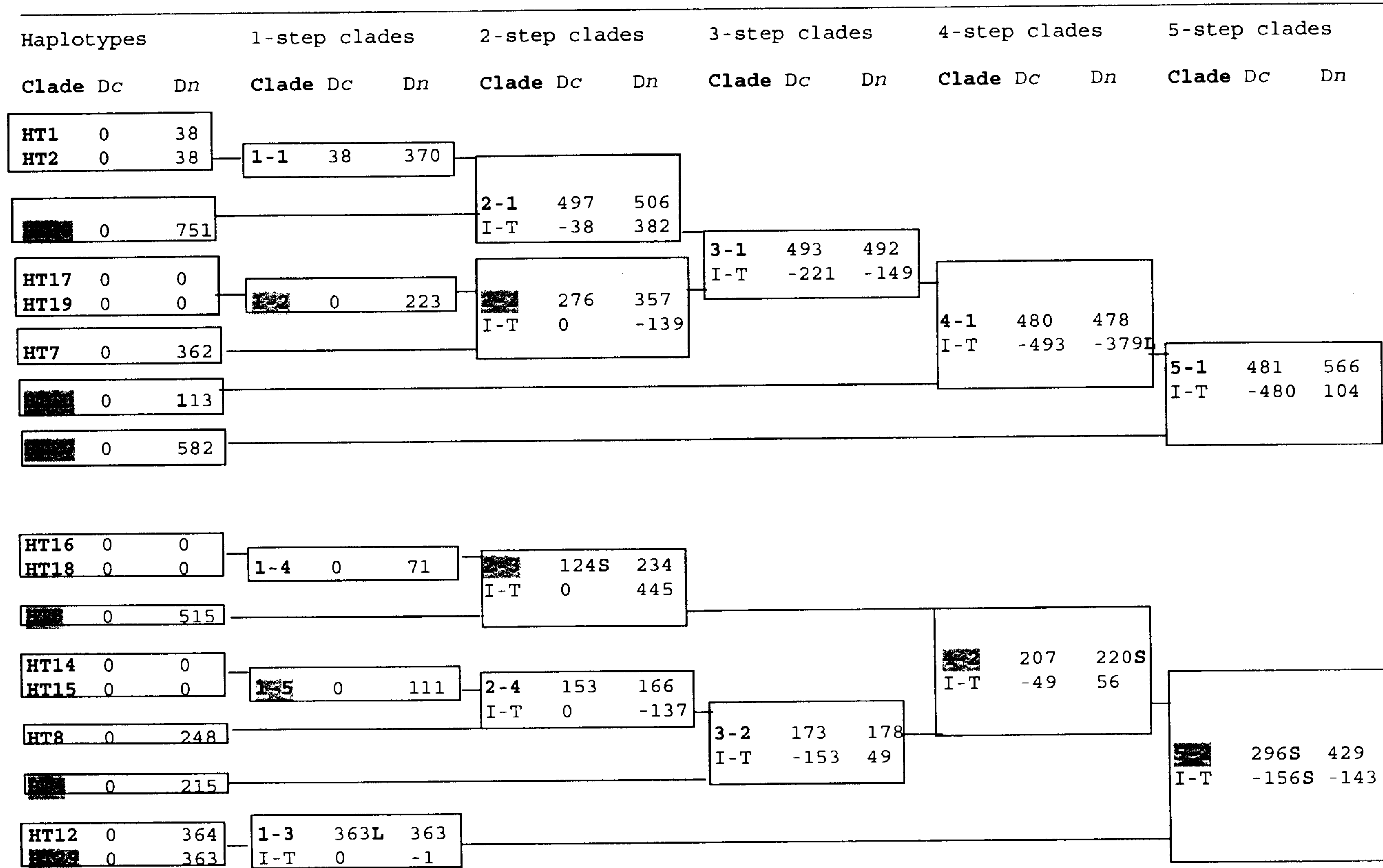
Table 6.7 Nested contingency analysis of geographic associations. Only clades with genetic/geographic variation are testable. The term "clade" here refers to the nesting clade. The permutational chi-square probabilities were calculated by 10 000 random permutations of the lower level clade categories within the nesting clade *versus* the geographical locality. Significant probabilities are in bold.

Clade	Permutational χ^2 statistic	Probability
1-1	4.00	0.329
1-3	2.00	1.000
1-8	5.00	0.404
2-1	5.00	0.198
2-2	3.00	0.336
2-3	10.00	0.106
2-4	6.00	0.068
2-5	5.00	0.101
2-6	6,00	0.500
2-7	4.00	0.342
2-8	3.00	0.330
3-1	8.00	0.101
3-2	1.56	0.436
3-3	0.60	1.000
3-4	8.00	0.144
3-5	**7.00**	**0.030**
4-1	3.94	1.000
4-2	**13.90**	**0.001**
4-3	6.65	0.121
4-4	5.24	0.176
5-1	10.00	0.204
5-2	**19.00**	**0.004**
5-3	9.03	0.090
Entire cladogram	**57.15**	**0.000**

Geographic Distance Analysis. Figure 6 illustrates the results of the analysis of the geographic distances revealing significant differences for several clade (D_c) and nested clade distances (D_n). Table 6.8 shows the interpretations of the significant results following the latest NCA key reference based on Templeton *et al.* (1995) and Crandall & Templeton (1996). There were no significant tests at the 1-step level. One of eight tests at the 2-step level was significant (2-5, Fig. 6.6), but the inference chain led to an inconclusive outcome (Table 6.8). At the 3-step level, two of five tests allowed for the usage of the inference key; two referential reports were retrieved for the 4-step level, and one at the 5-step level (Fig. 6.6). The global test for the entire network was also informative for describing the evolutionary dynamics responsible for the observed genetic variation. In most cases the inference chain indicated contiguous range expansion (CRE) as the likely evolutionary explanation for the observed association of haplotypes (Table 6.8).

Table 6.8 Inference chain for the results of the geographical distance analyses. Clade designations follow Fig. 6.5.

Clade	Chain of inference	Inference
2-5	1-2-11-17-NO	inconclusive outcome
3-4	1-2-11-12-13-14-NO	sampling design inadequate to discriminate between contiguous range expansion and long distance colonization
3-5	1-2-11-12-NO	contiguous range expansion
4-2	1-2-11-12-NO	contiguous range expansion
4-3	1-2-11-12-NO	contiguous range expansion
5-2	1-2-11-12-NO	contiguous range expansion
Total	1-2-11-12-NO	contiguous range expansion


Haplotypes
1-step clades
2-step clades
3-step clades
4-step clades
5-step clades
Clade Dc Dn
HT1 0 38
HT2 0 38
1-1 38 370
0 751
2-1 497 506
I-T -38 382
HT17 0 0
HT19 0 0
0 223
HT7 0 362
276 357
I-T 0 -139
3-1 493 492
I-T -221 -149
4-1 480 478
I-T -493 -379L
0 113
0 582
5-1 481 566
I-T -480 104
HT16 0 0
HT18 0 0
1-4 0 71
124S 234
I-T 0 445
0 515
HT14 0 0
HT15 0 0
0 111
2-4 153 166
I-T 0 -137
207 220S
I-T -49 56
HT8 0 248
3-2 173 178
I-T -153 49
0 215
296S 429
I-T -156S -143
HT12 0 364
0 363
1-3 363L 363
I-T 0 -1

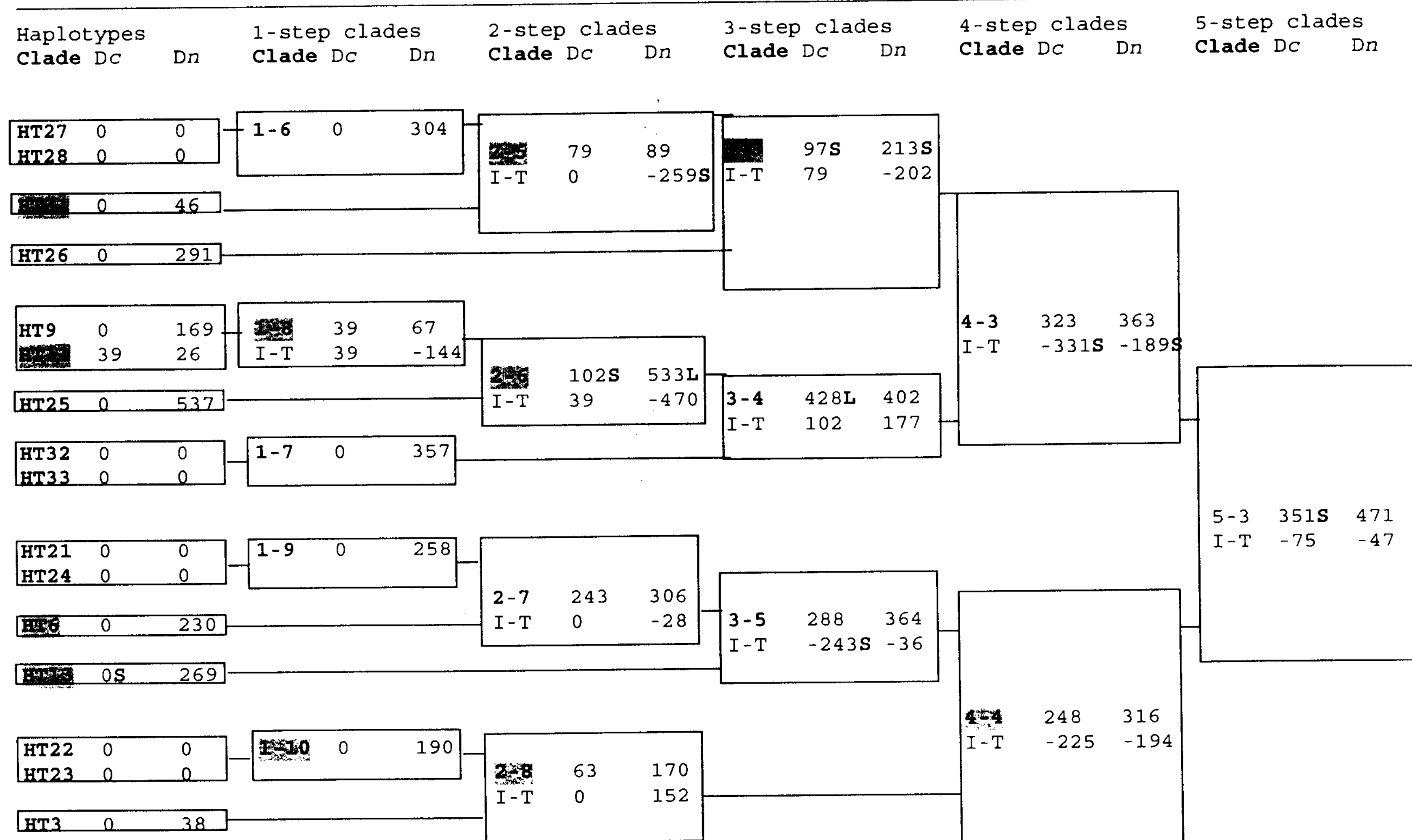


Fig. 6.6 Results of the nested geographic analysis of the Cape hare CR-I haplotypes. Haplotype designations follow Fig. 6.1 and Table 6.1. The nested design with the clade designations is given in Fig. 6.5. Clade (D*c*) and nested clade (D*n*) distances are given to the right of each haplotype or clade. Interior clades are shaded, interior *vs* tip contrasts for D*c* and D*n* are indicated as "I-T" in the corresponding nesting clade. The bold letters "S" and "L" indicate that the distance is significantly small or large, respectively, at the 5% level.

Microsatellites

HWE and genotypic disequilibrium. After Bonferroni correction, all observed genotype distributions for population SC were in HWE, despite the fact that this population had a small sample size (n = 13). For population N, only one locus (Lsa1) showed a significant ($P < 0.01$) deviation from HWE towards a heterozygote deficit (Appendix Table A10). This may have resulted from the presence of null alleles or substructured populations (Wahlund effect). Since no deviation from HWE was found at any one locus in more than one population, all loci were considered to be selectively neutral and were maintained for further analyses. None of 30 exact tests for pairwise linkage disequilibrium between the six loci was significant after Bonferroni correction (Appendix Table A11). Independent segregation of alleles at the six loci under investigation was therefore inferred, and all loci were retained for subsequent analyses.

Allelic variation of microsatellites. Forty alleles were found for the six microsatellite loci assessed herein (for compound genotypes see Appendix Table A9). All loci were polymorphic in each population (Appendix Tables A13-A18). The most variable locus was Lsa3 with 12 alleles over the whole sample; the least variable locus was Lsa2 with three alleles. The total number of alleles per locus per population ranged from two to 11. Locus-specific observed and expected heterozygosities (H_O/H_E) were lowest at Lsa2 (0.10/0.14) and highest at Lsa8 (0.77/0.74; Appendix Table A10). The population-specific values for mean H_O were 0.43 in SC and 0.50 in N, while mean H_E extended from 0.53 in SC to 0.56 in N. The frequencies of the most common alleles for each of the six loci varied between 0.35 and 0.95 (Appendix Tables A13-A18). One private allele was found in the SC group (at Lsa3), and 17 in the N population (over all loci except at Lsa6). All private alleles occurred at low frequencies (≤ 0.1).

Population structure. None of the six loci revealed significant differences in allele frequencies and only one locus (Lsa4) showed significant differences (at the $P < 0.05$ level) in genotype frequencies between SC and N (data not shown). Therefore the null

hypothesis of uniform allelic/genotypic frequencies for the pairwise population comparison could only be rejected for Lsa4.

The proportion of the genetic variation attributable to differences between the two populations quantified with the F_{ST} statistics revealed a substantial degree of differentiation ($F_{ST} = 0.1248$, $P < 0.001$). The R_{ST} value was smaller (0.0714), yet still reflected high genetic substructuring ($P < 0.05$). Pairwise R_{ST} values among the subspecies comprising clade N were non-significant (Table 6.9).

Table 6.9 Pairwise R_{ST} values (lower diagonal) and P values (upper diagonal) resulting from comparisons of the northern South African Cape hare subspecies.

	L. c. vernayi	*L. c. ochropus*	*L. c. ermeloensis*	*L. c. kalaharicus*
L. c. vernayi		0.56	0.28	0.49
L. c. ochropus	-0.0205		0.30	0.55
L. c. ermeloensis	0.0379	0.0201		0.31
L. c. kalaharicus	-0.0017	-0.0061	0.0210	

Genetic differentiation among the populations was also assessed calculating assignment tests in GENECLASS (Cornuet *et al.* 1999). The comparison of different assignment algorithms demonstrated that Nei's D (Nei 1972; based on the infinite allele model; IAM) outperformed those distance measures that rely on the stepwise mutation model (SMM; data not shown), corroborating Cornuet *et al.* (1999). Given the interrupted structure of repeat arrays in the loci used here, the mutational process is supposedly more similar to the IAM than the SMM. The Bayesian approach retrieved higher scores than the calculations based on Nei's DA (data not shown); however, the distance approach based on Nei's D led to the highest scores. Irrespective of the algorithm used, the "as-is" procedure always outperformed the "leave-one-out" procedure. We consequently chose the results of assignments obtained with the distance approach using Nei's D under the "as-is" procedure (Tables 6.10 and 6.11) for further analyses. The overall probability of correctly assigning animals to their population of origin based on their six-locus genotype was 58.21%, irrespective of whether the 21 sampling locations or the eight subspecies

were treated as distinct lineages. It increased to 82.09% under the hypothesis of two populations (as defined by the mitochondrial lineages SC and N; Table 6.10).

Table 6.10 Percentages of overall correct assignment achieved with Nei's Standard D (Nei 1972) under the "as-is" option (a-i) and under the "leave-one-out" option (l-o-o).

Hypothesis	Nei's D	Nei's D
	a-i	l-o-o
12 lineages	58.21	20.90
8 lineages	58.21	23.88
2 lineages	82.09	64.18

When testing the assumption of two distinct Cape hare populations, 76.92% of the animals collected in the south-central area were correctly assigned, and 83.33% of those drawn from the northern regions were correctly assigned (Table 6.12). Misassignments under the 12-lineage hypothesis were randomly distributed and also occurred across the SC-N border (data not shown).

Table 6.11 Assignment test results (Nei's D "as-is") in percent using the hypothesis of two distinct lineages based on the mitochondrially defined assemblages (SC = south-central and N = northern) in the Cape hare. The populations of geographic origin are given in the top row, and the assigned populations are given in the left column. The bold values along the diagonal show the percentage of correct assignments, n indicates the sample size.

Lineage	SC (n = 13)	N (n = 55)
SC	**76.92**	16.66
N	23.07	**83.33**

Phylogenetic reconstruction. The genetic distance as measured by Nei's D_A (Nei *et al.* 1983) between the two Cape hare populations was 0.055. The NJ tree derived from Nei's D_A (Fig. 6.7) showed random clustering of the sample localities; i.e. there was no correlation between the geographic positions of sample localities and their position in the tree. Generally, the bootstrap support values were very low (under 50%; Fig. 6.7). This was broadly consistent with the MIGRATE results based on the microsatelite data that indicated extensive gene flow between the two populations (9.9 migrants per generation from N to SC, and 11 from SC to N).

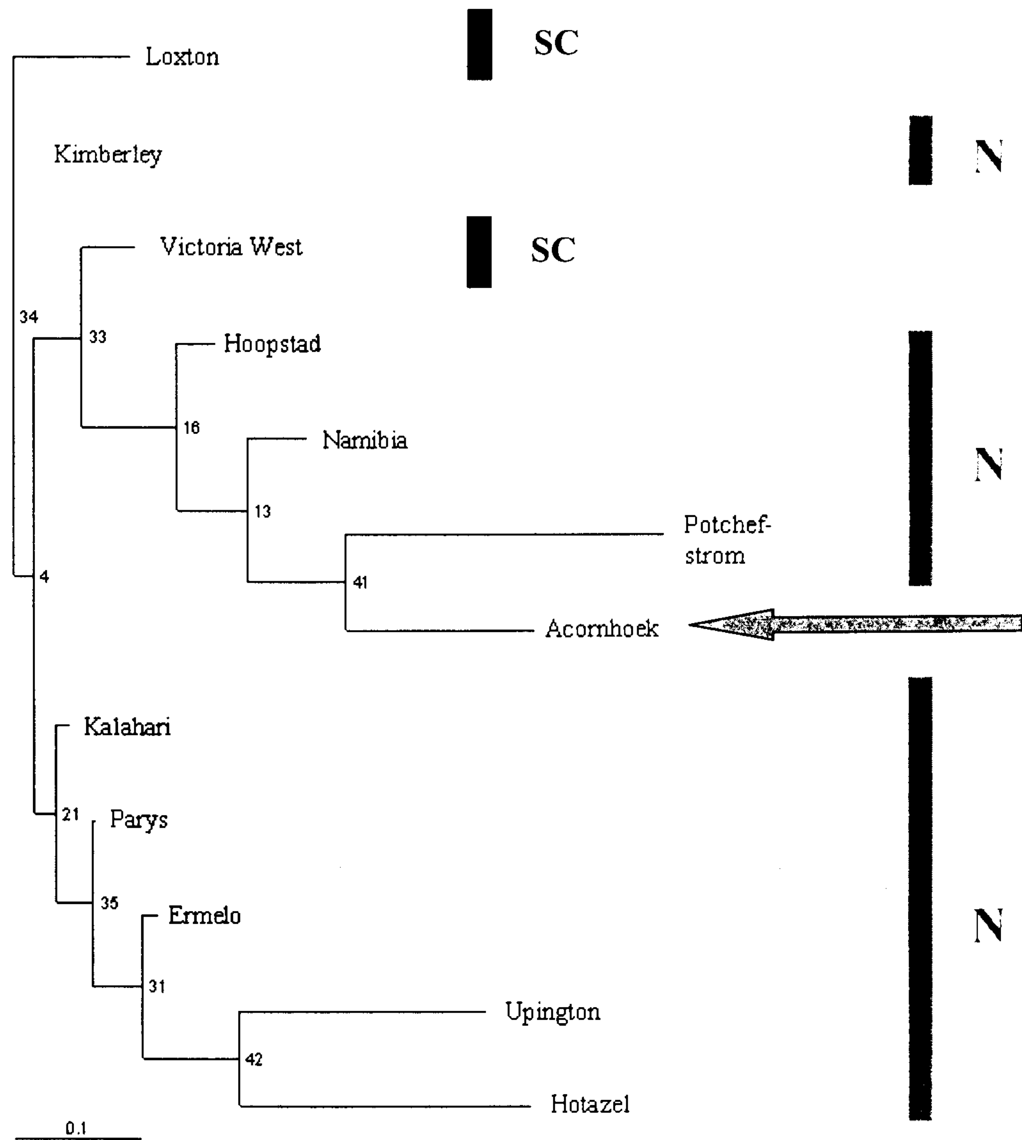


Figure 6.7 NJ phylogram based on Nei's D_A. The branch lengths reflect genetic distance; numbers at nodes show support from 1000 bootstrap replicates over loci. The bars at the right border indicate the mtDNA clade (SC = south-central and N = north). The arrow marks the animal from Acornhoek that possessed a very divergent mtDNA CR-I haplotype.

6.5 Discussion

Mutation rate and coalescence time

The estimated Cape hare CR-I mutation rate per nucleotide site per generation of $\mu = 3.56 \times 10^{-7}$ converts into a sequence divergence of 35.6% per million years (Myr). This value was considerably higher than the values proposed for humans (17.3% per Myr; Vigilant *et al.* 1991) or European hares (12.4% per Myr; Pierpaoli *et al.* 1999). However, it closely conformed to the CR mutation rates of 33% determined for humans (Ward *et al.* 1991) and 32% for scrub hares (Kryger *et al.* submitted; chapter 4).

Using this mutation rate, the time to the most recent common ancestor (TMRCA) for southern African Cape hares was estimated at approximately 230 940 years before present; this is very similar to the TMRCA calculated for the scrub hare (256 920 years before present) and places the appearance of Cape hares on the subcontinent at the mid-Pleistocene. This is consistent with the suggested appearance of the genus *Lepus* in Africa in the Pleistocene (Lavocat 1978; Caroll 1988) and the hypothesis of a rapid radiation of lagomorphs over all continents during the late Pleistocene (Angermann 1973; Chapman & Flux 1990). However, the calculations based on mtDNA may underestimate the coalescence time due to the reduced population size of this marker (Grant & Leslie 1993; Wakeley 2000).

Maternal lineages

The mtDNA CR-I variation in the South African Cape hare clearly identified two maternal lineages that were separated by a marked genetic discontinuity. The observed levels of genetic diversity were very high (Table 6.3). The haplotype diversities for the entire sample, as well as for the N group, were comparable to those found in the European hare, the mountain hare (Pierpaoli *et al.* 1999), and the southern African scrub hare (*L. saxatilis*; Kryger *et al.* submitted; chapter 4). The sequence divergence estimates between haplotypes within both assemblages were high, but only slightly higher than

what was found in scrub hares. The average number of nucleotide differences and the intrapopulational variability (Nei's D_X) were extremely high in both Cape hare populations; the values were approximately twice those obtained for the scrub hare. Correspondingly high levels of intraspecific mtDNA variation are reported for the European hare (*L. europeaus*; Perez-Suarez *et al.* 1994), and are thought to suggest that the species in question has persisted as a very large population for a considerable time.

The sequence divergence and Nei's net D_A between the two clusters were extremely high for an intraspecific comparison (roughly three times greater than the values retrieved for the scrub hare; Kryger *et al.* submitted; chapter 4) indicating a long-term historical separation of the two Cape hare lineages SC and N. The divergence time of 132 240 years ago (roughly twice that estimated among scrub hare populations) substantiated this idea. This date falls within the mid-Pleistocene, a period characterized by severe climatic fluctuations that presumably triggered similar diversification events in other South African mammals (Ewer & Cooke 1964, Grant & Leslie 1993). Importantly, there is no apparent zoogeographical barrier to dispersal that could account for the separation between the phylogeographic areas determined for the Cape hare. However, the SC lineage was restricted to the winter rainfall region of South Africa whereas the N assemblage occupied areas of the summer rainfall region. Approximately 130 000 years ago, the South African climate experienced a pronounced change to more arid conditions (Tyson & Preston-Whyte 2000). This probably opened up new habitat for the arid adapted Cape hare. As the climate returned to more humid conditions shortly after, it appears that the habitat may have fragmented again thus isolating the two populations. Alternatively, intrinsic barriers that may have included the socio-ecological behavior of the hares could have maintained the isolation between the populations (Mamuris *et al.* 2001 a,b).

While the differentiation between the two clades of Cape hares in southern Africa was pronounced (robust topology with consistently high bootstrap support values), the phylogenetic analysis failed to detect any phylogeographic structure within the assemblages. The sample size for the SC population was too small to allow further resolution. The N population was characterized by a star-like topology with short branch-lengths. This suggested that a range expansion occurred recently and not enough time has

elapsed yet for the haplotypes to sort by stochastic lineage extinction (Avise *et al.* 1984, 1990). Nigel & Avise (1986) noted that populations only approach reciprocal monophyly over a period of approximately four N_e (= effective population size) generations following their isolation. Using 132 240 years as the date of the isolation event and a generation time of two years (Marboutin & Peroux 1995) would predict that reciprocal monophyly would have been attained only if the effective population size for southern African Cape hares were 16 530 animals or less. All estimates of the female N_e alone calculated in this investigation were at least ten times higher.

Population history

The southern African Cape hare populations exhibited very high haplotype diversities in combination with low nucleotide diversities (4.9-5.9%; Table 6.3), which is indicative of a rapid population growth (Avise 2000). The mismatch distribution analyses (Figs. 6.3 and 6.4), the θ-values retrieved with three different methods (Table 6.7), as well as the negative values of three different neutrality statistics (Table 6.5) all suggested sudden population size expansions for the species in its entirety and the N population individually. Both expansion events were dated in the late Pleistocene, a time that coincides with the estimates of population increases in the European hare, the mountain hare (Pierpaoli *et al.* 1999), and the scrub hare (Kryger *et al.* submitted; chapter 4). The sudden ecological changes in southern Africa brought about by the Pleistocenic climatic oscillations (Tyson & Preston-Whyte 2000) are likely to have caused population expansions in this region. The θ-values (Table 6.6) were approximately twice those determined for the scrub hare. Since the mutation rate in both species was similar, it follows that the female historical population size in the case of the southern African Cape hare must have been twice that of the scrub hare.

The nested network constructed by following the nesting rules described in Templeton *et al.* (1987) and Templeton & Sing (1993) identified the two haplotypes from Loxton and Victoria West as one clade (1-1) which corresponds to the SC clade retrieved in the MP analysis. The NCA placed the divergent haplotype HT30 closer to SC than to the N group, suggesting that this haplotype may represent a third lineage of Cape hares.

Although no inferences could be made at the 1-step and 2-step levels, the overall picture emerging from the NCA for the Cape hare in southern Africa was characterized by contiguous range expansion as the driving evolutionary force. This result was more uniform, yet similar to the results obtained for the scrub hare (chapter 5). Moreover, it was consistent with our hypothesis of population expansion in response to palaeoclimatic change and a concomitant increase in habitat availability.

Population structure and gene flow inferred from microsatellites

The levels of microsatellite heterozygosity and allelic variation determined for the Cape hare were comparable to those found in the scrub hare (chapter 5) and in European lagomorphs (Surridge *et al.* 1999 a,b; Andersson *et al.* 1999). All but one Fisher's exact tests for among population differences in allele or genotype frequencies were negative suggesting a lack of nuclear genetic differentiation between the SC and N populations. In contrast however, significant F_{ST} and R_{ST} values indicated a moderate degree of population structure. Although both the F_{ST} and the R_{ST} values were significant the former was statistically more so, possibly due to the combination of moderate sample sizes and the low number of scored loci (Gaggiotti *et al.* 1999). The discrepancy between non-significant Fisher's exact tests but significant F- and R-statistics was similarly found in the scrub hare (chapter 5). Nonetheless, the assignment test results further emphasized the presence of population subdivision between the SC and the N clade: the scores of correct assignments were dramatically improved by reducing the number of source populations from 12 (sample localities) or eight (subspecies) to only two (mitochondrially defined clades). This was retrieved despite the fact that likelihood maximization (as used for the assignments in GENECLASS) intrinsically favors hypotheses with more parameters (source populations; see Dawson & Belkhir 2001) and therefore strongly supported the two-population-hypothesis. The nuclear genetic distance (Nei's D_A; Nei *et al.* 1983) between the two Cape hare populations was 0.055 – a value very similar to those calculated among three South African scrub hare populations (chapter 5; for pairwise Nei's D_A values among all sample localities see Appendix Table A19). The topology of the NJ tree based on Nei's D_A among sample localities

(Fig. 6.7) neither confirmed the phylogeographic partitioning into the SC and N assemblages, nor the differentiation among subspecies. The very low bootstrap values underscored the unreliability of the obtained topology. Although Nei's D_A is the most efficient distance measure in obtaining the correct tree topology (Takezaki & Nei 1996), the low number of loci used in this study seriously compromised the accuracy. According to Takezaki & Nei (1996), it is necessary to examine at least 50 loci in order to reach a probability of 88% for obtaining the correct topology. Moreover, it has recently been noted that some microsatellite loci can yield disproportionate interpopulational distance estimates and thus distort the assessment of evolutionary relationships among populations and different loci can contain incompatible phylogenetic information (Landry *et al.* 2002).

The ambiguous results obtained with different calculations based on the microsatellites may be caused by the systematic underestimation of differentiation with these markers due their high polymorphism (Hedrick 1999; Balloux *et al.* 2000). Furthermore, it is possible that male-biased dispersal, combined with female philopatry, led to a very pronounced partitioning of mtDNA variation while nuclear variation remained more uniform (Avise 1995, 2000). This pattern has been proposed for several lagomorph species (Webb 1988; Bell & Webb 1991; Webb *et al.* 1995; Surridge *et al.* 1999b; Reitz & Leonard 1994; this study chapter 5). The number of migrants between the two Cape hare populations per generation as calculated from the microsatellites was relatively high. Approximately 10 migrants per generation in both directions are sufficient to counteract genetic differentiation (Wright 1951). Preliminary results based on the mtDNA CR-I data retrieved less than one migrant per generation in both directions (data not shown). This would confirm the presence of gender-biased dispersal in the Cape hare.

Based on distinct mtDNA CR-I haplotypes and significant differentiation in nuclear microsatellite variation a deep phylogeographic partitioning was identified in southern African Cape hares, with one phylogroup occurring in the south-central part of the subregion and the other assemblage occupying the northern range. A strong phylogeographic structure has been reported for the South African rock hyrax (*Procavia capensis*; Prinsloo & Robinson 1992), for Smith's red rock rabbit (*Pronolagus rupestris*;

Matthee & Robinson 1996), and for the scrub hare (chapter 5); but the phylogeographic areas identified in these cases were different from those in the Cape hare. The reason for the lack of concordance between the Cape hare and the two rock-dwelling species (hyrax and red rock rabbit) may simply reflect different evolutionary histories due to differing habitat preferences. The difference in comparison with the scrub hare may be the result of an insufficient sampling regime in this study (no Cape hare specimens were obtained from the area where the SW scrub hare assemblage occurs).

Evolutionary distinctions

The distribution of animals comprising the SC assemblage seemed to correspond with the range attributed to *L. c. centralis*. The N group consisted of animals that comprised the distributional limits of seven distinct subspecies following Roberts (1951; see Table 6.1). It is possible that HT 30 (subspecies *L. c. bedfordi*) may represent a third distinct lineage since its sister-relationship to the N assemblage was not highly supported. Furthermore, this study did not incorporate specimens from the extreme southwestern areas of South Africa (subspecies *L. c. capensis*). No inferences concerning the evolutionary differentiation of the latter two subspecies can therefore be drawn without more extensive sampling. Within the N phylogroup, haplotypes did not cluster according to their subspecies categories: e.g. HT16-19 belonged to subspecies *L. c. ermeloensis*, but clustered on separate branches (Fig. 6.2). Moreover, HT11 and HT29 (both *L. c. hartensis*) were far apart in the tree topology. The same lack of cohesive geographic structure held true for the haplotypes of all other subspecies included in this investigation (*L. c. vernayi*; *L. c. ochropus*; and *L. c. kalaharicus*). In addition, animals from two different subspecies (*L. c. hartensis* and *L. c. ochropus*) shared haplotype HT11 (Table 6.1). AMOVA analyses also supported the partitioning of the South African Cape hares into two assemblages, thus further questioning the existing subspecies delimitations. In summary, our results do not support the distinction of *L. c. vernayi*, *L. c. ochropus*, *L. c. ermeloensis*, *L. c. kalaharicus*, and *L. c. hartensis*. The fur color and morphological differences between these subspecies are more likely to be adaptations to

climatic or environmental parameters than indicators of evolutionarily meaningful intraspecific subdivisions.

Based on the reciprocal monophyly at the mtDNA CR-I and the significant differences in nuclear allele frequencies, the recognition of the SC and the N clades as evolutionarily significant units (ESUs *sensu* Moritz 1994a, 1995) or even phylogenetic species (Cracraft 1989; Nixon & Wheeler 1990; Davis & Kevin 1992; Davis 1996) seems justified. There was no evidence for differences in allele frequencies at the mitochondrial locus or at the microsatellite loci among the subspecies from the N clade, and therefore no further differentiation into management units (MUs) was indicated.

Finally, this study represent a preliminary characterization of the genetic diversity of the Cape hares of South Africa only and does not allow extrapolations regarding the genetic population structure present in the species over the vast remainder of its presumed range. Further investigations over the complete range will be necessary to resolve the remaining questions concerning the exact geographic distribution and the intraspecific taxonomy of the Cape hare.

Chapter 7

How many species of *Lepus* in South Africa? – A molecular genetic perspective

7.1 Abstract

We used mitochondrial DNA sequences and microsatellites in combination with four morphological characters (ear length, breadth of the principal upper incisor, shape of the enamel invagination of the principal upper incisor, and the presence of a white spot on the forehead) to address long-standing controversies regarding the number and identity of species of hares (Genus *Lepus*) in South Africa. Phylogenetic analyses of 37 sequences of the hypervariable 5' end of the mitochondrial control region and 20 sequences of the entire cytochrome *b* gene unequivocally revealed the presence of four distinct maternal lineages among South African hares within two major clades. Significant F_{ST} and R_{ST} values as well as high assignment scores based on the microsatellite data of 227 southern African hares confirmed the nuclear genetic distinction of the four lineages. Applying a molecular clock, the separation of the included southern African hares from the European outgroup species occurred 4.84 million years ago (Ma) while that between the two South African clades occurred at 3.45 Ma. The divergence times between the subclades were 1.09–1.45 Ma. Based on the reciprocal monophyly in mitochondrial gene trees and the differences in allele frequencies at nuclear loci, the subclades warrant recognition as both evolutionarily significant units (ESUs), as well as phylogenetic species. The levels of the mitochondrial sequence divergences are consistent with the view of two species groups comprising two sister species respectively. The morphological characters did not allow a clear separation even between the two major clades and probably reflect common environmental pressures rather than a shared genepool.

7.2 Introduction

The taxonomic status of numerous species within the genus *Lepus* (Mammalia, Lagomorpha) is still uncertain (Angermann 1983; Chapman & Flux 1990) and at a more regional level, the evolution and phylogeny of African hares is particularly poorly known (Petter 1972; Angermann 1983; Angermann & Feiler 1988). The delimitation of hare species is not without difficulty, as they often resemble each other in morphology, behavior, and often occupy the same ecological niche (Angermann *et al.* 1990; Schneider 1990). Angermann (1983) and Flux & Angermann (1990) identified the scarcity of diagnostic morphological characters as a major constraint in leporid systematics. Widespread species show profound regional variation, with intraspecific variability often exceeding interspecific differentiation. Thus any taxonomy based on morphological traits such as pelage coloration is flawed, all the more so since pelage color varies with habitat and seasonal variations. Furthermore, due to the lack of morphological or karyological barriers, hybridization may occur easily between certain hare species and the hybrids are usually fertile (Angermann 1983; Flux & Angermann 1990; Thulin *et al.* 1997). Angermann *et al.* (1990) attributed these phenomena to *Lepus* being a relatively recent taxon with its radiation into the many recognized species being driven by open grassland habitat becoming more abundant through human agricultural and pastoral activities.

The number and identity of hare species recorded for South Africa has been controversial. Based on body size and coloration, Roberts (1951) listed two species: *L. capensis* Linnaeus, 1758 (the Cape or desert hare) and *L. saxatilis* F. Cuvier, 1823 (the bush or scrub hare). Petter (1972) identified a third species, *L. crawshayi* de Winton, 1899 (Crawshay's hare), for South Africa (thought to occur in Zululand and throughout the former Transvaal province) and determined the ear length as the distinguishing feature (> 130 mm = *L. saxatilis*, < 130 mm = *L. crawshayi*).

However, Robinson & Dippenaar (1983, 1987) applied univariate and multivariate morphometric analyses and could not find support for the presence of this taxon in South Africa. They considered *L. saxatilis* to be conspecific with *L. victoriae* (savanna hare), designating Petter's *L. crawshayi* and *L. whytei* as subspecies, and proposed that "*L. saxatilis*" should be used as the prior name for all South African scrub hares. In

accordance with Roberts (1951), they listed *L. capensis* and *L. saxatilis* as the only two species occurring in South Africa, arguing that the decrease in scull and ear length along a southwest-northeast axis in the scrub hare is clinal and therefore not indicative of different species. Skinner & Smithers (1990) and Duthie (1997) shared this view and specified the distribution of the scrub hare as extending from southwestern Mauritania and Senegal eastwards through most countries of West Africa and central and East Africa and over the whole of the southern African subcontinent.

In contrast, Angermann (1973 and 1983) treated *L. crawshayi* as a synonym of *L. whytei* Thomas, 1894, but clearly delineated *L. saxatilis* as a different species from *L. crawshayi*. This author suggested a difference in size and distribution between the two species; with the large *L. saxatilis* (southern bush hare, weighing between 3.5-7 kg) being restricted to the rocky mountain terrain of the southern half of South Africa and the medium sized *L. whytei* (African savanna hare, weighing 1.5-3 kg) distributed along West Africa all the way through to Kenya and Tanzania and over the whole of the southern subcontinent with the exception of the southern half of South Africa and central Africa. Flux & Angermann (1990) confirmed this view, but gave the name "*L. victoriae* Thomas, 1893" priority over "*L. whytei*" for the African savanna hare.

Kingdon (1974) accounted for two species in South Africa: *L. capensis* (brown hare) with a distribution similar to that mentioned by Angermann (1973) and *L. crawshayi* (Crawshay's hare) with a similar distribution like Angermann's *L. saxatilis* but reaching into the southern eastern part of South Africa. According to Kingdon (1974), it is close to impossible to tell the two species apart in the field, except for a white spot on the forehead being rare in the Cape hare but common in Crawshay's hare and the ears being longer in Crawshay's hare. Furthermore, the shape of a groove in the front of the principal upper incisors was suggested as a distinguishing feature between the two species: it was found to be shallow and without cement in the Cape hare but complex and filled with cement in Crawshay's hare (Kingdon 1974). Kingdon (1974) interpreted *L. whytei* as a close relative to *L. crawshayi* but with a distribution restricted to Malawi and Mozambique; he also acknowledged the existence of "a large drab coloured hare of uncertain affinities", *L. victoriae*, but did not give any distribution data. He speculated that *L. victoriae* might be a race of the Cape hare.

In contrast, Suchentrunk (1996) reported on a distinct separation of East African *L. capensis* and *L. victoriae* as two different species based on morphological distances. This author treated the labial enamel fold of the first upper incisor as diagnostic, with a simple pattern being characteristic for the Cape hare and a more complex pattern characterizing the savanna hare. Similarly, Robinson (1981, 1986) distinguished between South African *L. capensis* and *L. saxatilis* on the basis of these enamel patterns, again with the Cape hare showing the simple groove and the scrub hare showing a more complex groove. Duthie (1997) stated that the distinguishing character between the two species is the coloration of the underparts, being white in *L. saxatilis* and not white (but reddish or yellow-brownish) in *L. capensis*.

As is evident, all previous taxonomic hypotheses concerning South African hares were based mainly on morphological characters. These studies were either descriptive or relied on phenetics to group animals on cranial and dental characters. Chapman *et al.* (1990) identified the cladistic analyses of mitochondrial DNA (mtDNA) sequence data as one of the priorities for future research on lagomorphs; it was felt that the answer to the taxonomic difficulties within this group may lie more with nucleotide sequence variation than in anatomical features. With this as background, the aims of the present study were (i) to generate cytochrome *b* mtDNA sequence data for representative South African hare specimens, (ii) to analyze these sequence data with cladistic methods and to infer the number of distinct maternal lineages, (iii) to evaluate the differentiation of evolutionary lineages defined by nuclear variation among all available southern African hare specimens using data from six polymorphic microsatellite loci, (iv) to assess the taxonomic information of the characters ear length and shape of the anterior groove in the first upper incisor, and (v) to compare the results from the different genetic marker sets and superimpose them onto morphometric data.

Throughout this study we use the following terminology: *L. saxatilis sensu lato* (*s. l.*) when referring to the scrub hare in the sense of Robinson (1981, 1986); *L. saxatilis sensu stricto (s. str.)* when specifically referring to the genetically defined scrub hare of the southwestern Cape province of South Africa; *L. capensis s. l.* when referring to the Cape hare in the sense of Robinson (1981, 1986); *L. capensis s. str.* when specifically referring

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to the genetically defined Cape hare in the southern parts of South Africa. The reasoning behind this usage is explained in the discussion.

7.3 Materials and methods

Data Collection mtDNA

Samples and DNA extraction. Total genomic DNA extractions from 14 *L. capensis s. l.* and 21 *L. saxatilis s. l.* from different localities in southern Africa were utilized for the mtDNA part of this study. Out of a more comprehensive sampling scheme for population genetic studies (see chapters 4-6) we selected one Namibian representative per species, one scrub hare from Zimbabwe and the rest of 13 Cape hares and 19 scrub hares originated from 26 different localities throughout South Africa (Fig. 7.1) for this study.

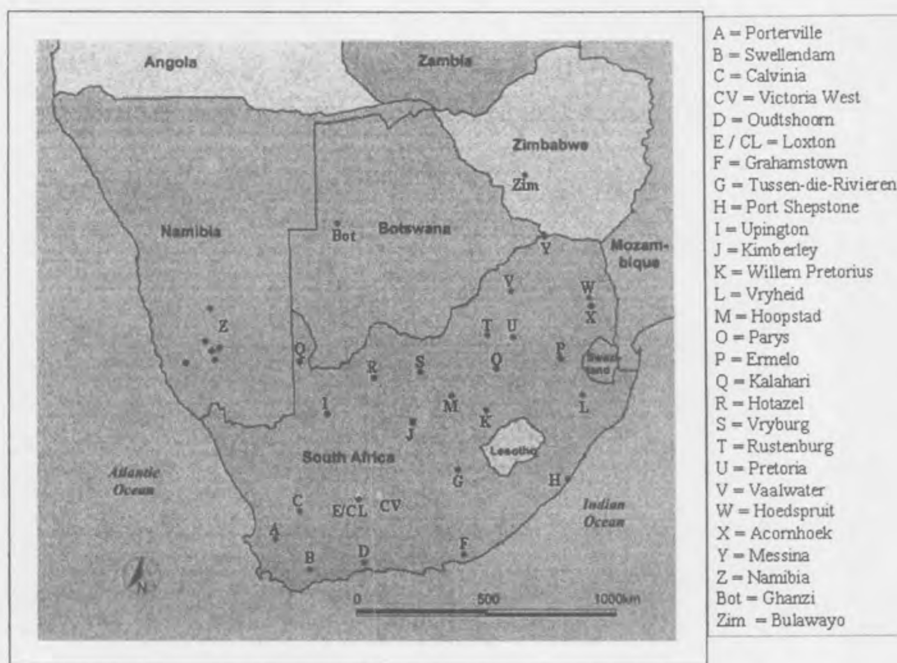

Fig. 7.1 Collection localities of the hare samples used in this study, Namibia (Z) was represented by several localitites that were in close proximity to each other.

MtDNA amplification and sequencing. The entire cytochrome *b* gene was amplified for eight Cape hares and eight scrub hares using the primers L 14724 (5' CGA AGC TTG - ATA TGA AAA ACC ATC 3') adapted from Pääbo *et al.* (1988) and H 15915 (5' GTC - ATC TCC GGT TTA CAA GAC 3') adapted from Irwin *et al.* (1991). The primer labels indicate the mitochondrial DNA strand (L = light strand and H = heavy strand) and the binding site of the 3' terminal base in the human mitochondrial genome (Anderson *et al.* 1981). Polymerase chain reaction (PCR) amplifications were performed in a 50 μl total reaction volume containing approximately 40 ng of the DNA extraction, 250 μM dNTPs, 1 mM $MgCl_2$, 1 μM of each of the two above listed primers and 1 unit of Super-Therm DNA polymerase (Southern Cross Biotech.). Amplification parameters for the cytochrome *b* fragment were as follows: one initial denaturing cycle for 5 min at 94 °C; 30 cycles of 30 sec denaturing at 94 °C, 30 sec annealing at 53 °C and 90 sec elongation at 72 °C; a final elongation step of 7 min at 72 °C. Each amplification experiment included a negative control to detect any contamination. All amplifications resulted in single, well-defined bands as visualized via agarose gel electrophoresis. Primers were removed from the amplification with the High Pure PCR product purification kit (Boehringer Mannheim). The amplified fragments were cycle sequenced in both directions in two overlapping segments with L14724 and the internal primer H15494 and with the internal primer L15408 and H15915 for cytochrome *b*. The BigDye sequencing kit was used and the cycle sequenced products were electrophoresed on an ABI 377 PRISM automated sequencer (PE, Applied Biosystems).

Additionally, we selected 14 representative Cape hare (*s. l.*) sequences and 21 representative scrub hare (*s. l.*) sequences from the mtDNA CR-I data sets from chapters 4 and 6.

Data collection microsatellites

See chapters 5 and 6.

Data analysis mtDNA

For each fragment the corresponding L- and H-primer sequences of each individual were aligned, compared and edited in SEQUENCE NAVIGATOR (PE, Applied Biosystems). The consensus sequences of all individuals were then automatically aligned in CLUSTAL X (Thompson *et al.* 1997).

Analyses to retrieve phylogenetic associations among lineages were performed in PAUP*4.0 beta 10 version (Swofford 2002). We ran a maximum likelihood ratio test as implemented in MODELTEST Version 3.04 (Posada & Crandall 1998) in order to determine the model of nucleotide substitution that fits the data most adequately. With a statistical significance of $P = 0.01$ the HKY85 model (Hasegawa *et al.* 1985) with gamma correction (Gu & Zhang 1997) and proportion of invariable sites obtained the best likelihood score for the cytochrome *b* data set; these parameters were selected for the neighbor-joining and the maximum likelihood analyses. The Ti/Tv ratio, the shape parameter (α) for the gamma distribution as well as the proportion of invariable sites were estimated using the maximum likelihood method (Felsenstein 1981) in PAUP.

GenBank sequences from the European hare (*L. europaeus* Pallas, 1778; Accession no AF149727; Fickel *et al.* 1999) and the Manchurian hare (*L. mandshuricus* Radde, 1861; Accession no AJ287975; Wu & Zhang, unpublished data) were used as outgroup taxa for the CR I based analyses. We utilized GenBank sequences from the Italian hare (*L. corsicanus*; Accession no AF157464; Pierpaoli *et al.* 1999), the mountain hare (*L. timidus*; Accession no AF157466; Pierpaoli *et al.* 1999), the Spanish hare (*L. granatensis*; Accession no AF157465; Pierpaoli *et al.* 1999) and the Manchurian hare (*L. mandshuricus*; Accession no AJ279422; Wu & Zhang, unpublished data) as outgroups in our analyses of the cytochrome *b* data.

Gene trees of both mtDNA data sets (cytochrome *b* and CR-I) were constructed with the neighbor-joining (NJ) algorithm (Saitou & Nei 1987) based on the pairwise distances and using the option of randomized tie breaking. For the parsimony analysis (Hennig 1966; Felsenstein 1982 & 1988), a randomized input order was activated and the alignment gaps in the CR I data set were treated as a fifth character state and coded following Simmons & Ochoterena (2000) in order to account for the insertions/deletions

present among members of the ingroup taxa. Nodal support for the trees was assessed by 1000 (NJ and MP) or 100 (ML) bootstrap iterations (Felsenstein 1985). In the case of the cytochrome *b* derived MP tree, we used the computer program AUTODECAY version 4.0 (Eriksson 1998) to determine the number of extra steps required to disrupt monophyletic clades as described by the decay index (DI; Bremer 1988). Since down-weighting transitions or third codon positions did not change the topology, the phylogenetic analyses of the cytochrome *b* data were performed without any weighting scheme. This was done in accordance with Broughton *et al.* (2000) who suggested that transitions provide more phylogenetic information than transversions in protein-coding mitochondrial genes, and should thus not be down-weighted. The relative age of nodes in a molecular based phylogenetic tree can be estimated from the genetic distances between the operational taxonomic units (OTUs) united by this node. This is done by assuming that the number of nucleotide substitutions between taxa is directly proportional to the time elapsed. The assumption of such a strict molecular clock, however, is only legitimate if there is no variation in mutation rates among lineages (Barraclough & Nee 2001). In order to test for rate heterogeneity among the different lineages in this study, we performed a log-likelihood ratio test (Hasegawa & Kishino 1994) and compared the log-likelihood scores (*ln*L) of the cyt *b* and CR-I MP trees with and without the enforcement of a molecular clock (Felsenstein 1988).

Data analysis microsatellites

The six loci used here were shown to be selectively neutral, in Hardy-Weinberg equilibrium and not linked (see Results in chapters 5 and 6).

A neighbor-joining tree was constructed in POPULATIONS Version 1.2.26 (Langella 1999-2002) using Nei's genetic distances, D_A (Nei *et al.* 1983; Takezaki & Nei 1996) for all pairwise comparisons (with the sample localities representing the OTUs), and 1000 replicates bootstrap support for the nodes were calculated. The tree was displayed and saved as a graphic file in the computer application TREEVIEW (Page 1996).

In order to test for structuring of the nuclear genetic variation among the mitochondrially defined lineages, we performed Fisher's exact tests for differentiation

(Raymond & Rousset 1995b; Rousset & Raymond 1997) and estimated Wright's fixation index, F_{ST} (Wright 1951, 1965; Weir and Cockerham 1984), and the unbiased multilocus R_{ST} statistics (Slatkin 1995). Additionally, assignment tests were run as implemented in the program GENECLASS (Cornuet *et al.* 1999).

Morphometric data

The breadth of the first upper incisor and the length of the ears were measured for all individuals where possible. Furthermore, the shape of the anterior groove of the principal upper incisor was documented and the presence/absence of a white spot on the forehead was recorded. These characters were chosen, because of their previous implications in the taxonomy of South African *Lepus* (Petter 1972; Robinson 1986). The coding for a simple groove in the principal upper incisor was "1", for a complex groove "2". Likewise, the absence of a white spot on the forehead was coded with "1", and the presence with "2 ".

The computer programme STATISTICA (Stasoft Inc. 1995) was used for the analyses of the four morphological variables. As *a posteriori* analysis, discriminant function analysis was performed to find the combination of variables that would best discriminate between the individuals under investigation and to maximize the variation between the defined groups while minimizing the variation within them. The groups were defined by the genetic lineages found within South African hares. *A priori* multivariate analyses included the unweighted pair-group arithmetic average (UPGMA) cluster analysis based on Euclidian distances and principal component analysis (PCA) based on product-moment correlation coefficients among variables (Sneath & Sokal 1973). Cluster analysis allows for the grouping of individuals into hierarchical clusters beginning with those that are most similar. Principal component analysis combines variables into a smaller number of factors that are linear combinations of the original variables and that allow two-dimensional representation of the results in scatter plots. The partitioning of the genetic variation was then superimposed onto the results of these statistics in order to determine whether the four morphological variables would reliably distinguish between some or all of the genetically defined lineages.

7.4 Results

MtDNA

Control Region. The CR-I data set comprised 420 aligned positions of which 157 (37%) were variable (Table 7.1) and 130 (31%) were parsimony informative. Five positions included insertions/deletions (positions 45, 46, 158, 163, and 219; Table 7.1). The maximum likelihood estimated Ti/Tv ratio was 6.6, the gamma shape parameter indicated rate variation ($\alpha = 0.622$) and the proportion of invariable sites was 0.25. Phylogenetic reconstruction retrieved the same topology, irrespective of the algorithm used (Fig. 7.2). The MP analysis recovered 163 best trees with a length of 374 steps (consistency index = 0.476; retention index = 0.818) and placed the European brown hare (*L. europaeus*) as more basal in the outgroup than the Manchurian hare (*L. mandshuricus*). It further identified two major monophyletic clades that were supported by high bootstrap values, one consisting entirely of *L. saxatilis s. l.* and one consisting exclusively of *L. capensis s. l.*. The average sequence divergence between these two clades was extremely high (approximately 38%). Within the "saxatilis" clade, high support was retrieved for the monophyly of the southwestern group, *L. saxatilis s. str.* (Fig. 7.2). One animal, Z07 from Namibia, clustered basal within this clade and was separated by 9.8-12.3% sequence divergence from all other scrub hares, while the mean sequence divergence between the two subclades was 7.99% (Table 7.2). The average sequence divergence among animals within the southwestern subclade was 1.86%; it was 5.62% within the northern subclade. Within the "capensis" clade, two subclades were supported with over 90% bootstrap values – the south-central group, *L. capensis s. str.,* and the northern group. Intersubclade average sequence divergence was very high (25.82%; Table 7.2). The intrasubclade mean divergences were 13.35% in the south-central group and 7.41% for the northern cluster. One animal, X01 originating from Acornhoek, did not belong to either subclade and showed sequence divergences of approximately 23% in comparison with any other Cape hare. Sequence divergences between ingroup and outgroup taxa were extremely high (40-55%) and lay at

approximately 26% between the two outgroup species (Table 7.2). For pairwise sequence divergences among all included specimens see Appendix (Table A5).

Cytochrome b. The primers for the amplification of the cytochrome *b* gene annealed within flanking, highly conserved transfer RNA genes. Furthermore, the amplified sequences comprised an open reading frame without insertions or deletions or stop codons and the translated amino acid sequences (see Appendix Table A7) were sufficiently similar to those published of other leporids (Pierpaoli *et al.* 1999; Fickel *et al.* 1999). Therefore it was inferred that the amplification products had originated from functional cytochrome *b* genes rather than nuclear pseudogenes. The cytochrome *b* data set comprised 1020 aligned positions (Table 7.3; for complete alignment see Appendix Table A6) of which 266 (26%) were variable and 194 (19%) were parsimony informative. The base composition was strongly biased towards a scarcity of G (only 5.67%), which is typical for mammalian cytochrome *b* sequences (Johns & Avise 1998). The maximum likelihood estimated Ti/Tv ratio was 11.3. The gamma shape parameter (α = 1.29) indicated a small range of varying rates and the proportion of invariable sites was 0.567. Phylogenetic reconstruction under three different algorithms (NJ, MP, and ML) resulted in congruent topologies (Fig. 7.3). The MP analysis retrieved 12 best trees with a length of 372 steps (consistency index = 0.575; retention index = 0.811). The Italian hare and the mountain hare grouped as sister species, which was in agreement with Pierpaoli *et al.* (1999). As with the CR-I data, the reciprocal monophyly of *L. capensis s. l.* and *L. saxatilis s. l.* was highly supported. The average sequence divergence between these two clades was 13.8% (for all pairwise sequence divergences see Appendix Table A8). The subdivision of the "saxatilis" clade into a southwestern and a northern subclade (average intersubclade sequence divergence 4.37%, Table 7.2) was also supported with high bootstrap values. The same held for the subdivision of the "capensis" clade into a south-central and a northern clade (average intersubclade sequence divergence 5.8%; Table 7.2). All these clusters were supported by Bremer's decay indices with values greater than four (Fig. 7.3). The animal X01 from Acornhoek clustered outside the two major Cape hare clades, as was found with the CR-I topology, and was separated by 6.98% sequence divergence from all other Cape hares. Sequence divergences among

ingroup and outgroup species mostly ranged from 14-24%, except the value of 3.75% between the two outgroup sibling species *L. corsicanus* and *L. timidus* (Table 7.2). For pairwise sequence divergences among all included specimens see Appendix (Table A8). The observed sequence divergences were well in the range reported for leporid cytochrome *b* sequences (Halanych & Robinson 1999; Halanych *et al.* 1999; Pierpaoli *et al.* 1999).

The likelihood ratio test indicated rate heterogeneity among the different lineages of the CR-I data set: the log-likelihood score for the tree without a molecular clock enforced was $-\ln L = 2302.81614$, whereas the log-likelihood score for the tree with an enforced molecular clock was $-\ln L = 2381.79471$. The difference in likelihood scores was highly significant (78.97857; 35 degrees of freedom; $P < 0.001$) and consequently the hypothesis of a molecular clock had to be rejected for the CR-I data. In contrast, the cytochrome *b* sequences were found to evolve at constant rates over the investigated lineages: the log-likelihood score of the tree without an enforced molecular clock was $-\ln L = 3489.92118$, whereas the value with the clock enforced was $-\ln L = 3501.05980$. The difference in likelihood scores was not significant (11.13862; 18 degrees of freedom; $P > 0.1$), and consequently it was not possible to reject the hypothesis of a molecular clock for the cytochrome *b* data.

Table 7.1 Variable sites defining the haplotypes of 37 OTUs for 420 bp of the CR-I. L. sax. = *Lepus saxatilis s. l.*, L. cap. = *L. capensis s. l.*, L. man. = *L. mandshuricus*, L. europ. = *L. europaeus*, SW = southwestern assemblage, C = central assemblage, N = northern assemblage, SC = south-central assemblage. The coding of the geographic localities follows Fig. 7.1.

	1111111111111111111111111111111122
	11111111222222222333333334444455556778889990001111222333334455566666689999901
Taxon/Node	37801234579012346789012356785678901564690132380290234079015677808901234720236911
L.sax.A02	CTTACATATCAGTACCTGTCACTATGAC--CCTTTTGAATTTTACTTTCTTTTTTAGATCTTGG-CCTTATCTTCCCTTG
L.sax.B01	..............................T...
L.sax.C01	....
L.sax.D01	............................A..........C.....C..T.C.C......G.C.................A
L.sax.D02	......................................GC.....C..T.C.C......G.C.................A
L.sax.F02	....C........T...CC.....G.C................CA
L.sax.G01	............................A...............TC..T...C......G.C.A................
L.sax.H01	....T.......................A...................T...C......G.C.A................
L.sax.J06	....C........T...CC.....G.C................CA
L.sax.K01	T...........................A................C...C..C......G.C..................
L.sax.K05	T...........................A................C......C......G.C..................
L.sax.L01	............................A................C..T...C......G.C..................
L.sax.L06	............................A................C.CT..........G.C..................
L.sax.S01	T...........................A................C......C......G.C..................
L.sax.T01	............................A...............TC.CT...C......G.C.A................
L.sax.U06	T...........................A................C......CC.....G.C..................
L.sax.V03	T...........................A................C......C......G.C..................
L.sax.W01	............................A................C..T..C.......G.C...T........T.....
L.sax.Y02	T..C......C......G.C..................
L.sax.Zim1	TC....C......................................C.........GA..G.C........A..AA....A
L.sax.Z07	T.......C...................A..........C.....C...C..C......A.C..................
L.cap.I01	..G...CG.A....T.CA..........TC.A...CACGCC.......TA.....CAC.A.C.AA....G....A.TAA.
L.cap.J01	..A...CG.A....T.C...........TC.A...CACGCC......C.A..C..CAC.A.C.AA....GCT.CA..GA.
L.cap.J03	..G...CG.A....T.CA..........TC.A...CACGCC........A.....CAC.A...AA....G....A.TAA.
L.cap.M01	..G...CG.A....T.C...........TC.A...CACGCC......C.AC....CAC.A.CAAGT...G...CA..AA.
L.cap.N01	..G...CG.A....T.C...........TC.A...CACGCC...T...TA.....CAC.A...AA.......C.A..AA.
L.cap.O01	..G...CG.A....T.CA..........TC.A...CACGCC.......TA.....CAC.A...AA....G....A..AA.
L.cap.P01	..G...CG.A....T.CA..........TC.A...CACGCC........A.....CAC.A...AA.........A..AA.
L.cap.P05	..G...CG.A....T.CA..........TC.A...CACGCC........A.....CAC.A...AA.........A..AA.
L.cap.Q08	..G..GC..A....T.C...........TC.A...CACGCC........A.C...CAC.A...AAT........A.TAA.
L.cap.R01	..G...CG.A....T.C...........TC.A...CACGCC.......TA.....CAC.A...AA.........A..A..
L.cap.Z05	..G...CG.A....T.C...........TC.A...CACGCC........A.C...CAC.A...AAT........A.TAA.
L.cap.X01	..A...CG.A...................TT.A..CACGC.CAT.CC..A......AC.A.C.AA...CT...CT..AA.
L.cap.CV1	..A...C..A..................TC.A..CCAC.C....A...TAA.C.CCACCA...AA..C.G.......GC.
L.cap.CL1	..A...C..A..................TC.A..CCACGC.C......TAA.C.CCACAA.CAAA....G..C..T.G..
L.man.	..ACA.ATCAGTAC.TCTCACTATGACATT..CC.C..GCC.......TC.....GAC.A.C.AA.......C....A.A
L.europ.	T.ACATATCAGTA..TGTCACTATGACACA..C.....GC......C.TC.....GAC.ACC..G.TC.-...C...A..

Table 7.1 continued.

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             22222222222222222222222222222222222222222222222233333333333333333333333333334
             11112222222233333334444444555556666667777788899900011112223344556666777777782
Taxon/Node   27890123456903567890145678135690234580145834512905912450788904022349013457820
--------------------------------------------------------------------------------------------
L.sax.A02    TTCAACTGTTCCTCTAATCCTTCAACCCAATCATATATACCTATCCCCCTTTCATGCAATTCTTTCTCCAGGGCCGT
L.sax.B01    C.T.......T...........................TC.....................CT..........
L.sax.C01    ..T...................................TC.....................C...........
L.sax.D01    C......A.CT......C.T..T....TG..T.C....T.TC.....T.........G......CT..........
L.sax.D02    C......A.CT........T..TG...TG..T.C....T.TC.....T......C..G......CT..........
L.sax.F02    C......A.C.............G...TG..T.C.C.CT.TC....TT................C...........
L.sax.G01    C....T.A.CT................G..T.C.C..T.TC.......................CT.....A.....
L.sax.H01    C.T..T.A.CT.............T.TG..T.C.C.CT.TC.......................C......A.....
L.sax.J06    C......A.C..C..........G...TG..T.C.C.CT.TC....TT................C...........
L.sax.K01    C....T....T...................T......T.TC.....T.....G..........C...........
L.sax.K05    C....T....T...................T......T.TC.....T.....G..........C...........
L.sax.L01    C....T.A.CT.C....C.........T...TTC...CT.TCG...TT.C...R..........CY.Y...R....Y
L.sax.L06    C....T.A.CT................TG..T.C.C.CT.TC......................C...........
L.sax.S01    C....T....T...................T......T.TC.....T.................C...........Y
L.sax.T01    C....T.A.C.......C.........TG..T.C.C.CC.TC......................C......A....Y
L.sax.U06    C....T....T...........T........T......T.TC.....T.....G..........C...........Y
L.sax.V03    C....T....T...................T.C....T.TC.....T.................C...........
L.sax.W01    C....T.A.CT.C..............T...TT....CT.TCG...TT.C...G..........C...........
L.sax.Y02    C....T....T........T..........T......T.TC.....T.....G..........C...........C
L.sax.Zim1   CC....C........T.C.....T...............TC.....T.C...............C......AA....
L.sax.Z07    C........CT......C.T.........G.T.C....T.TC.....T................C...........
L.cap.I01    .AA.CT...A.TCTA.CCTTGCT......C.T.AG..C..T.....T..CACTGAA..C..T..CT.TT..AA.TAA
L.cap.J01    .AAGCT..CATTC.A.TC.TGC....T..C...AG..C..TC.....T.CACTGAA..C.....CT.TT.AAATTAA
L.cap.J03    .AA.CT...A.TC.A.CC.TGCT...TT.C.T.AG..C..TC.....T.CA.TGAA..C.....CT.T...AA.TAA
L.cap.M01    .AA.CT...A.TC.A.CC.T......T..C...AG..C..TC.....T.CACTGAA..C.....CT.TT.AAATTAA
L.cap.N01    .AA.CT...A....A.CC.TG.T....T.C...A......TC..T..TT.ACTGA...C..T..CT.TT..AA.TAA
L.cap.O01    .AA.CT...A..C.A.CC.TGCT...TT.C.T.AG..C..TC....TT.CA.TGAAT.C.....CT.T...AATTAA
L.cap.P1     .AA.CT...A....A.TC.TG.T....T.C...A....G.TC.....T.CACTGA...C.....CT..T...ATTAA
L.cap.P5     .AA.CT...A....A.TC.TG.T....T.C...A....G.TC.....T.CACTGA...C.....CT..T..AATTAA
L.cap.Q08    .AA.C....A..C.A.CC.TG.T...TT.C...AG..C..TC....TT.CA.TGAAT.C..T..CT.TT...A..AA
L.cap.R01    .AA.CT.A.A....A.CCTTG.T....TGC.T.A......TC.....T.CACTGA...C..T..CT.TT..AA.TAA
L.cap.Z05    .AA.CT...A..C.A.CC.TGCT...TT.C...AG..C..TC.....T.CA.TGAAT.C..T..CT.TT..AAT.AA
L.cap.X01    ....CTCTCA.TC.A..C.T.A......TC.T.AC...GTTC...TTT.CA..GA...C..T..C..TT..A..TAW
L.cap.CV1    C...TT.ACA..C.A..C.T.C.......T.T.AC..CG.TC.....A.CA...A...CC.TC...C.TGAA..TAA
L.cap.CL1    C.T.CT.ACA....A..CT...TG.....C.T.AT...G.TC....TA.CA...A...C.CTA.CT.TTG.A..TAA
L.man.       ..T-C...C.A...A.C....C..C.....C..C.ACAT..C.C...T.CG...A...C.C.CCCT.TT..AA....
L.europ.     ....C..AC.A......CT.....C..TG..T.AT..C...C.......CG...AA.GC..T..C.....TAA.TA.
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Table 7.2 Average pairwise sequence divergences in percent (HKY85 Γ-corrected) among the four mitochondrially defined lineages of southern African hares and the five outgroup species computed using control region I (CR-I; above the diagonal) and cytochrome *b* sequences (below the diagonal). Ls = *Lepus saxatilis s. l.*, Lc = *L. capensis s. l.;* SW = southwestern, CN = central-northern, N = northern, and SC = south-central; Lman = *L. mandshuricus*, Leur = *L. europaeus*, Lcor = *L. corsicanus*, Ltim = *L. timidus*, Lgra = *L. granatensis.*

	LsSW	LsCN	LcSC	LcN	Lman	Leur	Lcor	Ltim
LsSW	-	7.99	34.96	35.12	41.2	41.03	-	-
LsCN	4.37	-	33.61	37.51	48.33	43.57	-	-
LcSC	15.76	15.27	-	25.82	55.14	52.92	-	-
LcN	17.09	14.09	5.8	-	48.22	53.50	-	-
Lman	22.76	22.37	21.35	23.92	-	25.36	-	-
Lcor	22.08	20.51	19.74	20.26	23.03	-	-	-
Ltim	18.30	17.0	18.03	19.49	20.89	-	3.75	-
Lgra	17.95	17.35	21.93	20.58	17.96	-	16.76	15.97

Table 7.3 Variable sites defining the haplotypes for the 1020 bp long fragment of cytochrome *b* of the 20 OTUs. L. sax. = *Lepus saxatilis s. l.*, L. cap. = *L. capensis s. l.*, L. man. = *L. mandshuricus*, L. grana. = *L. granatensis*, L. timid. = *L. timidus*, L. corsic. = *L. corsicanus*, SW = southwestern assemblage, C = central assemblage, N = northern assemblage, SC = south-central assemblage, Achk = Acornhoek. The coding of the geographic localities follows Fig. 7.1.

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                                     111111111111111111111111222222222222222222222222333333333333333333
                   12223334556677889900000112223445555667778900011123333344556677788900011122222334445 56
Taxon/Node      3698457016548692814062358947036214034605479921470692245783658143695873692581457869258789
--------------------------------------------------------------------------------------------------------
L.sax.A         AATACCATCCTTGACCTACTCCTCCTCCTTATAACTATCTGCGTCCTTCCATATTATTACAACTCCAATAACCATTTCTCCCCCTCCG
L.sax.B         ...........C..........................T.................................................
L.sax.D         .G.....C..CC.........T................T.A..C.........CC.....G......G.GG................A
L.sax.E         .G.....C..CC.........T................T.A..C.T.......CC............G.GG................A
L.sax.T         .......C..CC.........T................T.A..C.........CC............G..G................A
L.sax.Y         .......C..CC......T..T................T.A..C.........CC............G..G................A
L.sax.Zim       .......C..CC.........T................T.A..C.........CC............G..G................A
L.sax.Z         .......C..CC.........T................T.A..C.........CC............G.GG..............T.A
L.cap.J         ..C...GC.TCC.GT.C.T.....T.T..C.C......T.A..C....A..C..CG.C.A.G.....GA.T..........TT.C..A
L.cap.M         ..C...GC.TCC.G..C.T.....T.T..C.C......T.A..C....A..C..CG.C.A.G.....GA.T..........TT.C..A
L.cap.N         ..C...GC.TCC.G..C.T.....T.T..C.C......T.A..C....A..C..CG.C.A.G....GGA.T..........TT.C..A
L.cap.O         ..C....C.TCC.GT.C.T.....T.T..C.C......T.A..C...CA..CG.CG.C.A.G.....GA.T..........TT.C..A
L.cap.Q         ..C....CTTCC.G..C.T.....T.T..C.C......T.A..C....A..C..CG.C.A.G.....GA.T..........TT.C..A
L.cap.CV        ..C......T.C....C.T.....T.T..CT....CGCT.A..C.T.CA..C..CGCCGA...C....A.T..G........T....A
L.cap.CL        ..C......T.C....C.T.....T.T..C...G..GCT.A..C.T.CA..C..CGCC.A...C....A.T..G........T....A
L.cap.X         ..C.A..C.TCC.G..C.T..T..T.T..C......G.TCA..C.T..TT.C..CG.C.A...C...GA.C...C......TT...TA
L.corsic.       ..C...GC.TC...TTC.TC..CT..TT.C.C..T..CT.AT.CTT.CA.G...CTC.....T.....A.CTT......TT......A
L.grana.        T.C..TGC.T.C....CTTC.......TCCG.T.....TCA..C.TC.A....CC........C.T..A..T.G....C....TC.TA
L.timid.        ..C....C.TCC..TTC.T....T..TT.CGC..T..CT.AT.C.T.CA.G...C.C.....T.T...A.CTT..C...TT......A
L.mandsh.       .G.GA..C.T..A...C.TCT....CT.C.G......C.CA.AC...CA.G..CCGCC.......T..A..T....CTC.....CT.A
```

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                3333333344444444444444444444444444455555555555555555555555555555555555666666666666666666
                7778899901222333444555666667888899900111112233344445566666777888888999001122233446667778
Taxon/Node      2481403954069235147349235897034925817036895814703692814567039023589789390814703580392581
--------------------------------------------------------------------------------------------------------
L.sax.A         CGGATTCCTCTCCTCCTCCACAAACTTTCTGTCCTCACTGCACTATACCCCTCATGTTAAAAACCTCTATCCTCTCTTACCCCCTCTC
L.sax.B         ........................................................................................
L.sax.D         ........C....C.............C...............C.....TT........G.G..T.......C.....C.........
L.sax.E         ........C....C.............C...............C.....TT........G.G..T.......C.....C.........
L.sax.T         ........CT...C.............C...........A...C.....TT....A...G.GT.T.......C.....C.........
L.sax.Y         ........CT...C.............C...........A...C.....TT....A...G.GT.T.......C.....C.........
L.sax.Zim       .........T...C.............C...........A...C.....TT........G.GT.T.......C.....C.........
L.sax.Z         .........T...C.............C...............C.....TT........G.G..T.......C.C...C.........
L.cap.J         ..A..C..A..T.C.A...T.......C...CTT.T.ACA..T..C.T..T.T....C..T.C.TC.C.CT......CCT.....T..
L.cap.M         ..A..C..A..T.C.AC..T.......C...CTTCTGA.A..T..C....T.T....C..T.C.TC.C.CT......CCT.....T..
L.cap.N         ..A..C..A..T.C.A...T.......C...CTT...A.A..T..C.T....T..C.C..T.C.TC.C.CT......CCT.....T..
L.cap.O         ..A.....A..T.C.A...T.......C...CTT.TGACA..T..C.T..T.T....C..T.CGTCTC.CT......CCT.....TC.
L.cap.Q         .....C..A..T.C.A...T.......C...C.T.TGACA..T..C.T..T.T....C..T.C.TC.C.CT......CCT.....T..
L.cap.CV        ..A..C..A....C.A..T........C...CTT.T.ACA.....C.T..T.T.C..C..T.C..C.G..T......CC...T..T.T
L.cap.CL        ..A..C..A...TC.A..T........C...CTT.TGACA.....C....T.T.C..C..T.C.TC.G..T......CT...T..T.T
L.cap.X         ..AT.C..A...TCTAC.T.......CC....TT..GACA.....C.T..T.T.C..C..C.C.TC....T......CCT.TT..T..
L.corsic.       T.AGCC.TC.......C....GG.TCCC.......T.TCA..T.G.....TCT.CC....CG..TC.C.......T.C.T.TT.CTCT
L.grana.        ..AG..T........TCTT.....TC..T.........CA..T.....T..CT..C.TGT..T..C.......T...CC.TT..CTC.
L.timid.        T.A..C.TC.......C...TGG.TC.C......C..TCA..T.G.....TCT.CC....CG..TC...........C...TT..TC.
L.mandsh.       .CA...T...A..C.AC.T..C.G.C.C.CA..TC...CAGG....C.T..C.GCACC..T.C.AC..C..TC..TAC..TT.T.T..
```

Table 7.3 continued.

```
                                                                                                         1
              666667777777777777777777777777777888888888888888888888888999999999999999999999999999999990
              899990111222223334455556667788999012233444555566666778999000112223333344455566666777889999
Taxon/Node    403692147013692581403492584779056495717039258901457395147039281470367925814803693581434691
------------------------------------------------------------------------------------------------------------
L.sax.A       CTGGTCCTCCTACCTCCGATTACATATCGCACTCCATCCCTCTCGGCCTGCTCCACAAACAATCTCAAGAACTCCTCTCTCCGCCAACGC
L.sax.B       ..........................................................................................
L.sax.D       ...A.....T........T..C.........T......T.C...A.T.C......T...T..............................
L.sax.E       ...A.....T........T..C.........T......T.C...A.T.C......T...T..............................
L.sax.T       ...A.....T........T..C.........T......T.C...A.T.C......T...T..............................
L.sax.Y       ...A.....T........T..C.........T.T....T.C...A.T.C......T...T..............................
L.sax.Zim     ...A.....T........T..C.........T.....TT.C...A.T.C......T...T..............................
L.sax.Z       ...A.....T........T..C.........T......T.C...A.T.C......T...T.G............................
L.cap.J       T.AAC.....C..T.T.AT..C....C.A..TC.T..T...T..AATTC..CTT.T...T..CTCT.....TC.T..CT.T.AT......
L.cap.M       T.AAC.....C..T.T.AT..C....C.A..TC.T..T...T..AATTC..CTT.T...T...TCT.....TC.T..CT.T.AT......
L.cap.N       T.AAC.....C..T.T.AT..C....C.AT.TC.T..T......A.TTC..CTT.T...T...TCT.....TC.T..CT.T.AT......
L.cap.O       T.AAC.....C..T.T.AT..C....C.A..TC.T.CT...T..A.TTC..CTT.T...T..CTCT.....TC.T..CT.T.AT......
L.cap.Q       T.AAC.....C..T.TTAT..C....C.A..TC.T..T...T..AATTC..CTT.T.G.TG..TCT.....TC.T..CT.T.AT......
L.cap.CV      T.AA......C.TTCT.AT..C.....TA..TC..G.T...T..A.TTC..CT..T...T...TCT..A..TC.T..CT.T.AT....A.
L.cap.CL      T.AA..T.T.C.TT.T.AT..C.....TA..TC..G.T...T..A.TTC..CT..TG..T....C...A..TC.T..CT.T.AT......
L.cap.X       T.AAC.....C..T.T.AT..C.....TA.TTC.T..T...T..AATTC..CT..T..GT...TCT.GA.GTC.T..CT.T.AT.G....
L.corsic.     ..A..T.C.T....CT.A.C.C...G..AT.........TC.C.AATTC.TCG.GT...T...T.T.....TCT..T..C.TATT..T.T
L.grana.      ..AAC....T.G..CTTA.CCC.G...TATT...T...TTCT..AAT.C.TCTTG....T.C...T.....A.T.A.C.C.TA...G...
L.timid.      ..A..T.C.T....CT.A...C......AT........GTCTC.AAT.CATCA.G....T...T.T.....TC...TC...TA.T..TAT
L.mandsh.     .CAAC..C.T..TTCT.A...CT.C.CTAT.........TC..TAAT....CG..T.G.T...T.TT..G.TCT...CTC......G.AT
```

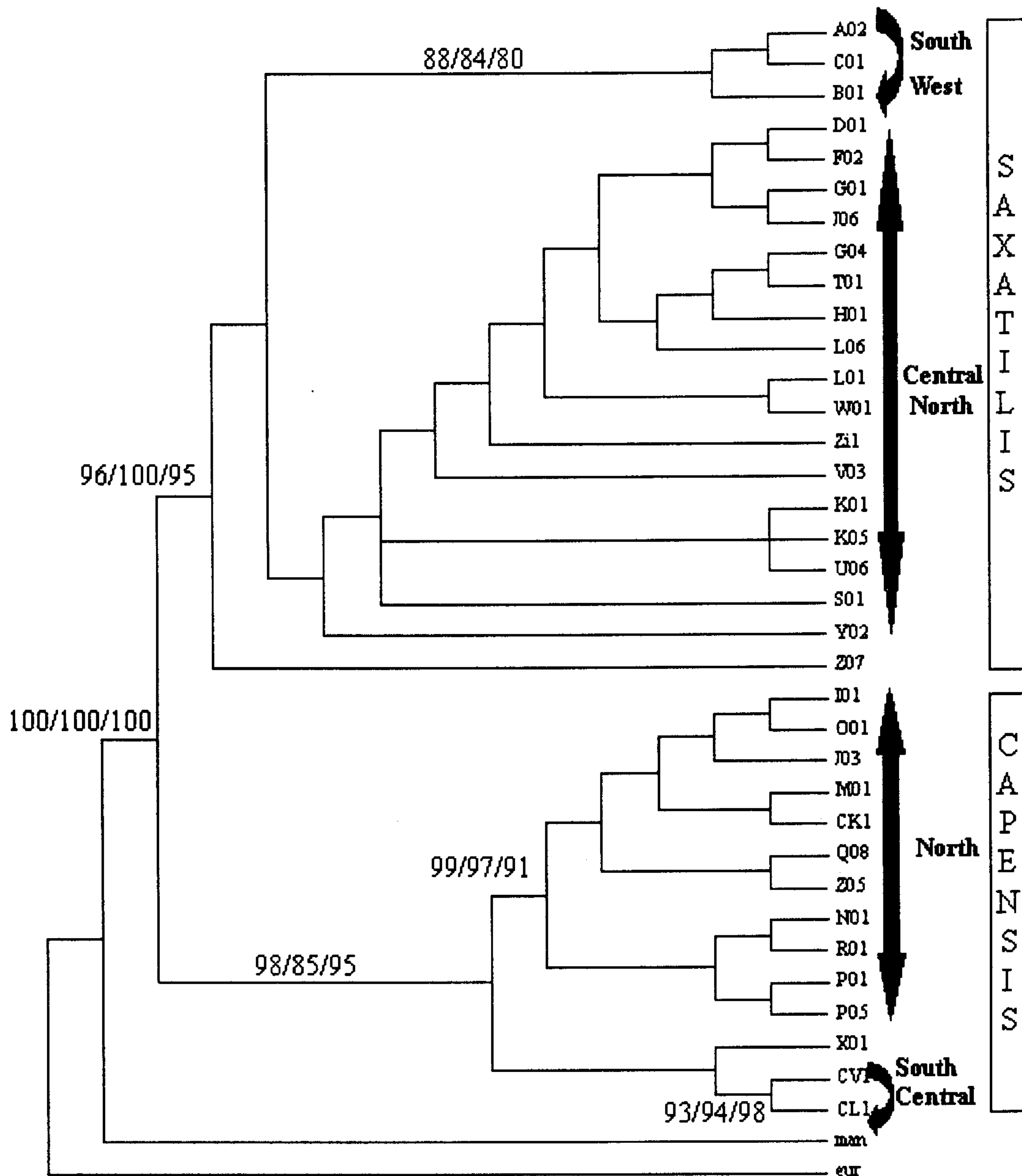

Fig 7.2. Phylogenetic tree based on 420 bp of CR I sequences. Taxa labels follow Figure 7.1. All three analyses (NJ, MP and ML) produced congruent topologies. Values above the branches indicate the bootstrap support after 1000 replicates (NJ/MP/ML).

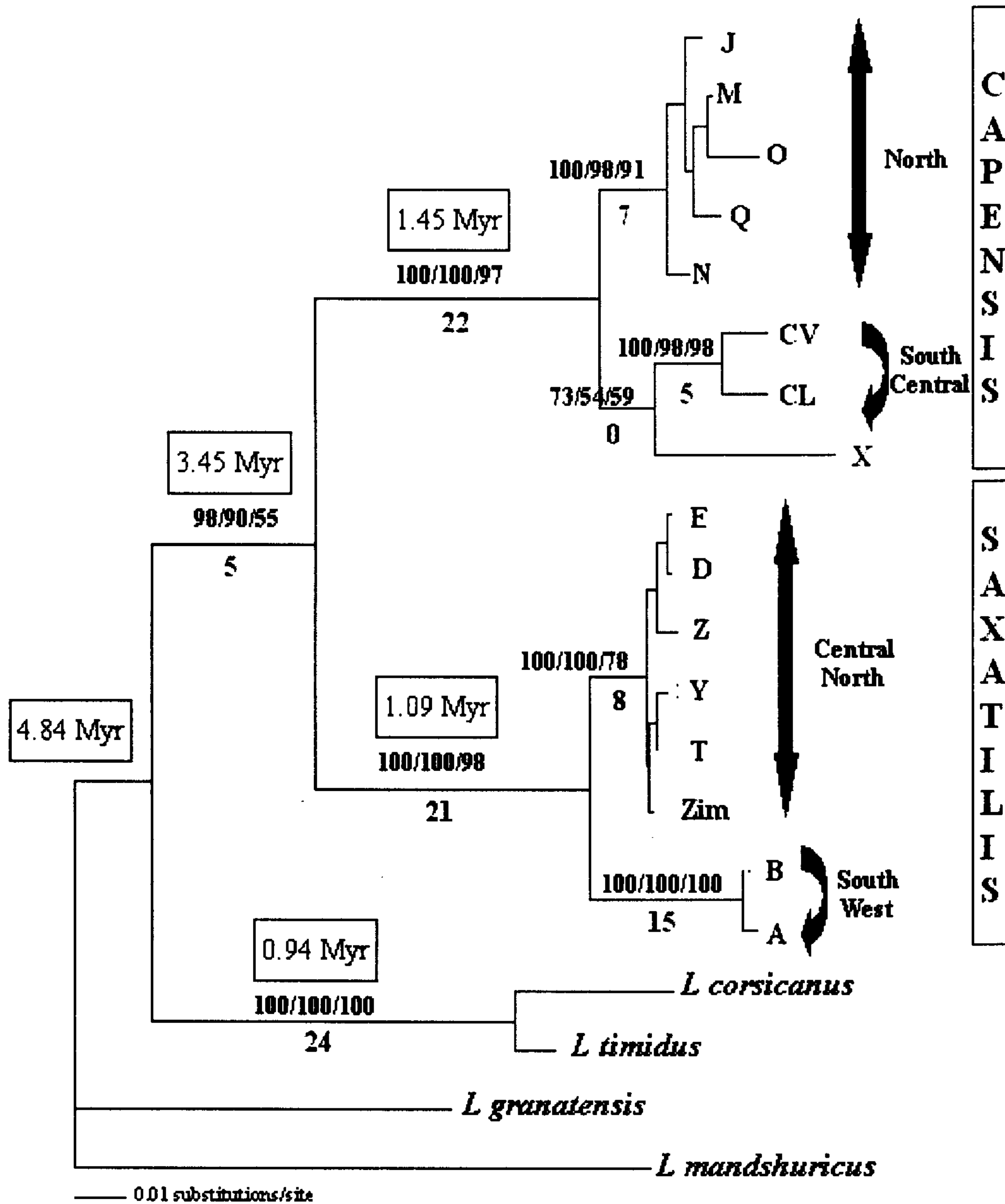

Fig 7.3 Phylogenetic tree based on 1020 bp of cytochrome *b*. Taxa labels follow Figure 7.1. All three analyses (NJ, MP, and ML) produced the identical topology. Values above the branches indicate the bootstrap support after 1000 replicates (NJ/MP/ML), values beneath the branches indicate Bremer's decay index (DI) values. Numbers in boxes refer to the calculated divergence times.

Microsatellites

The topology of the neighbor-joining tree constructed from the microsatellite data was not well supported (most bootstrap values < 50%) and did not retrieve the mitochondrially defined lineages as monophyletic clusters (Fig. 7.4).

The results of the Fisher's exact tests consistently supported the distinction between *L. saxatilis s. l.* and *L. capensis s. l.* (Table 7.4). The nuclear genetic variation between the southwestern scrub hares and the northern Cape hares was also highly significant at the genic and genotypic level for most loci. All other pairwise comparisons failed to produce consistently significant results (Table 7.4).

All pairwise F_{ST} and R_{ST} values were highly significant and thus indicative of genetic differentiation (Table 7.5). There was a tendency for the R_{ST} values to be larger than the F_{ST} values (especially pronounced in the overall mean values), which suggested a relatively ancient differentiation among the defined groups (O'Ryan *et al.* 1998).

The assignment tests performed better using the model-based Bayesian method in comparison to the distance-based methods and also under the "as-is" procedure compared to the "leave-one-out" procedure (Table 7.6). All further conclusions were therefore based on the Bayesian "as-is" results. The scores obtained under the four-lineage hypothesis were higher than 75% supporting this subdivision (Tables 7.6 and 7.7).
Nuclear genetic distances between the two major clades measured in Nei's DA were 4-5 times higher than the within-clade distances (Table 7.8; Appendix Table A19).

Table 7.4 Results of Fisher's exact tests for genic/genotypic differentiation between *Lepus saxatilis s. l.* (Ls) and *L. capensis s. l.* (Lc) and among the four mitochondrially defined lineages (Ls = scrub hare, Lc = Cape hare; SW = southwestern, CN = central-northern, N = northern, and SC = south-central). The significance levels are indicated as * $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, n.s. = not significant.

Locus	Ls - Lc	LsSW – LsCN	LsSW – LcN	LsSW – LcSC	LsCN – LcN	LsCN – LcSC	LcN – LcSC
Lsa1	***/***	***/***	n.s./n.s.	n.s./***	***/n.s.	*/*	n.s./n.s.
Lsa2	***/***	n.s./n.s.	***/***	n.s./***	**/*	n.s./*	n.s./n.s.
Lsa3	***/***	n.s./n.s.	***/***	n.s./***	***/n.s.	*/n.s.	n.s./n.s.
Lsa4	***/**	n.s./n.s.	**/**	n.s./**	***/n.s.	n.s./n.s.	n.s./*
Lsa6	***/***	n.s./n.s.	***/***	***/***	***/***	***/***	n.s./n.s.
Lsa8	***/***	**/**	*/**	n.s./***	***/n.s.	n.s./n.s.	n.s./n.s.

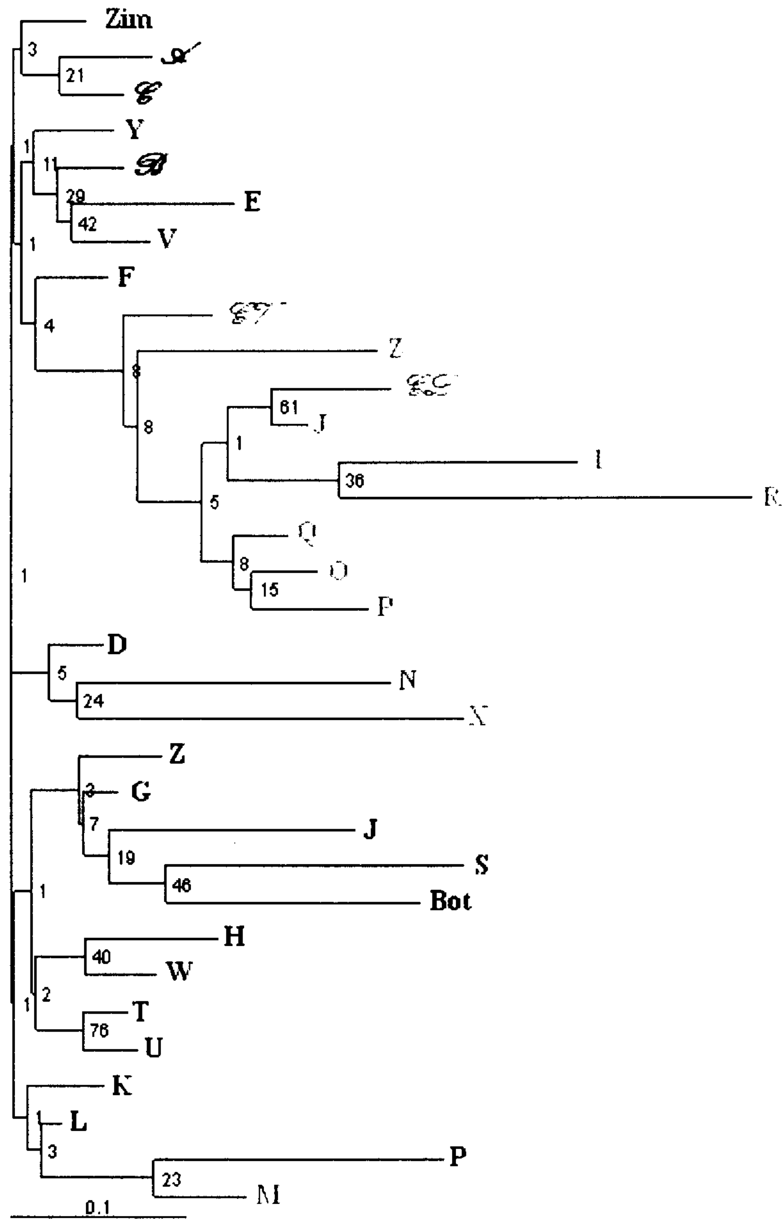

Fig 7.4 Neighbor-joining tree derived from Nei's D_A (Nei *et al.* 1983) calculated from six microsatellite loci in POPULATIONS (Langella 1999-2002). The values beneath the branches represent the bootstrap support after 1000 replicates over loci. Black letters code for central-northern scrub hares, black "Kunstler script" letters code for the southwestern scrub hares, gray letters code for northern Cape hares, and gray "Kunstler script" letters code for south-central Cape hares.

Table 7.5 Total and pairwise F_{ST} (upper diagonal) and R_{ST} values (lower diagonal) as calculated by GENETIX and RSTCAL, respectively; significance levels after 10 000 permutations indicated in parenthesis as * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, n.s. = not significant, (Ls = scrub hare, Lc = Cape hare; SW = southwestern, CN = central-northern, N = northern, and SC = south-central).

	LsSW	LsCN	LcSC	LcN	total
LsSW		0.0951 (***)	0.0972 (*)	0.1072 (***)	0.0814 (***)
LsCN	0.07820 (**)		0.1196 (***)	0.1207 (***)	
LcSC	0.24282 (***)	0.16955 (***)		0.1248 (***)	
LcN	0.23169 (***)	0.20089 (***)	0.10596 (**)		
total	0.18326 (***)				

Table 7.6 Percentages of correct assignments calculated when genotypes were grouped respectively into 33, four, or two different clades. Analyses were based on three different approaches implemented in GENECLASS - the model-based Bayesian analysis, and distance-based analyses using either Nei's DA (Nei *et al.* 1983) or Nei's Standard D (Nei 1972, 1978). Each of the three analyses was tested under the "as-is" option and the "leave-one-out" option.

Hypothesis	Bayesian	Bayesian	Nei's DA	Nei's DA	Nei's D	Nei's D
	as-is	l-o-o	as-is	l-o-o	as-is	l-o-o
33 lineages	55.61	13	47.53	11.66	46.64	16.14
4 lineages	75.78	65.47	66.82	58.3	69.51	62.78
2 lineages	95.96	92.83	93.27	92.38	92.38	91.93

Table 7.7 The results of assignment tests (Bayesian, "as-is") given in percentages for the hypothesis of four distinct lineages (Ls = scrub hare, Lc = Cape hare; SW = southwestern, CN = central-northern, N = northern, and SC = south-central). The populations of origin plus the sample size (n) are given in the top row, the assigned populations are given in the left column. The diagonal in bold shows the percentage of correct assignments.

Assemblage	LsSW (n=27)	LsCN (n=129)	LcSC (n=13)	LcN (n=54)
LsSW	**77.7**	18.6	0	0
LsCN	18.5	**75**	7.6	1.9
LcSC	3.7	4.6	**84.6**	24
LcN	0	1.5	7.6	**74**

Table 7.8 Nuclear genetic distance (Nei's D_A) between the four mtDNA defined hare lineages (Ls = scrub hare, Lc = Cape hare; SW = southwestern, CN = central-northern, N = northern, and SC = south-central).

	LsSW	LsCN	LcSC	LcN
LsSW		0.044	0.290	0.223
LsCN			0.244	0.183
LcSC				0.055

Morphology

It was not possible to consistently distinguish between *L. saxatilis s. l.* and *L. capensis s. l.* by the means of any of the four morphological characters. The white spot on the forehead was present in some but not all scrub hares as well as Cape hares (Appendix Table A1). The shape of the enamel invagination on the anterior surface of the principal upper incisor was simple in all Cape hares but complex in many, yet not all, scrub hares. While only 7.69% of the southwestern scrub hares (n = 26) possessed a complex groove, 63.89% of the skulls investigated from the northern group (n = 108) showed this feature (Appendix Table A1). The breadth of the principal upper incisor ranged from 1.3-3.0 mm with an average of 2.17 mm in the Cape hare (n = 50) and from 1.8-3.5 mm with an average of 3.1 mm in the scrub hare (n = 136); the length of the ears showed a similar overlap (92-121 mm in 48 Cape hare specimens and 92-151 mm in 135 scrub hares; Table 7.9). However, the variation of the latter character separated the southwestern lineage from the central-northern one within the larger scrub hare cluster, and the south-central lineage from the northern one within the larger Cape hare cluster (Table 7.9).

Table 7.9 Measurements of the breadth of the principal upper incisor and the ear length in millimeters for the four genetic lineages of hares in South Africa.

Genetic lineage	Breadth of upper incisor (mm) Average/Range	Ear length (mm) Average/Range
L. saxatilis SW	3.0/2.6-3.4	137.5/126-151
L. saxatilis CN	2.7/1.8-3.5	110.7/92-150
L. capensis SC	2.0/1.3-2.5	116.1/108-121
L. capensis N	2.3/1.8-3.0	103.2/92-109

The discriminant function analysis resulted in a dispersion matrix where the determinant equaled zero. Since this occurred due to traits inherent in our data set, it was not possible to continue with this analysis (Chris Chimimba, personal communication).

The cluster analysis revealed two major clusters, one of which (A) comprised *L. saxatilis s. l.* (Fig. 7.5). Cluster B contained all *L. capensis s. l.*, but interdispersed with scrub hares from the CN group which indiscriminately clustered throughout the entire dendrogram. The two Cape hare groups clustered separately (the south-central group in cluster C and the northern group in cluster D) with the exception of three individuals of uncertain placement (Fig. 7.5).

The PCA extracted four principal factors (Table 7.10), the first three of which explained 92.05% of the variance. The first principal component accounted for over 37% of the total variance and corresponded to breadth of the principal upper incisor and the ear length. The second principal component explained nearly 32% of the total variance and represented mainly the shape of the anterior groove in the principal upper incisor.

The analysis of the first two principal components clearly separated the southwestern scrub hare lineage from both Cape hare lineages (Fig. 7.6). Within the scrub hares, it was not possible to discern between the southwestern and the central-northern groups. Only after subdividing the latter into its two subpopulations (C and N; see chapters 4 and 5 for details), emerged a pattern – the SW and the N groups occupied mutually exclusive areas in the scatter plot, but the C assemblage overlapped with both. The analysis further allowed the unambiguous demarcation of the C and N scrub hares from the south-central Cape hares. However, the separation between the northern Cape hares and the C and N scrub hares was not complete (Fig. 7.6). Furthermore, there was a considerable overlap in the areas occupied by the two Cape hare lineages.

The additional incorporation of component 3 into the analysis did not result in any further separation among the groups (data not shown).

Table 7.10 Loadings on the principal components for each of the four morphological variables; in bold are the loadings > 0.700.

Variable/Percent of total variance	PC 1/37.74	PC 2/31.92	PC 3/22.39	PC 4/7.95
Breadth of principal upper incisor	**-0.792**	0.482	0.139	0.348
Shape of groove in upper incisor	0.204	**0.825**	0.463	-0.252
Ear length	**-0.910**	-0.162	-0.117	-0.364
White spot on forehead	0.113	0.582	**-0.805**	-0.032

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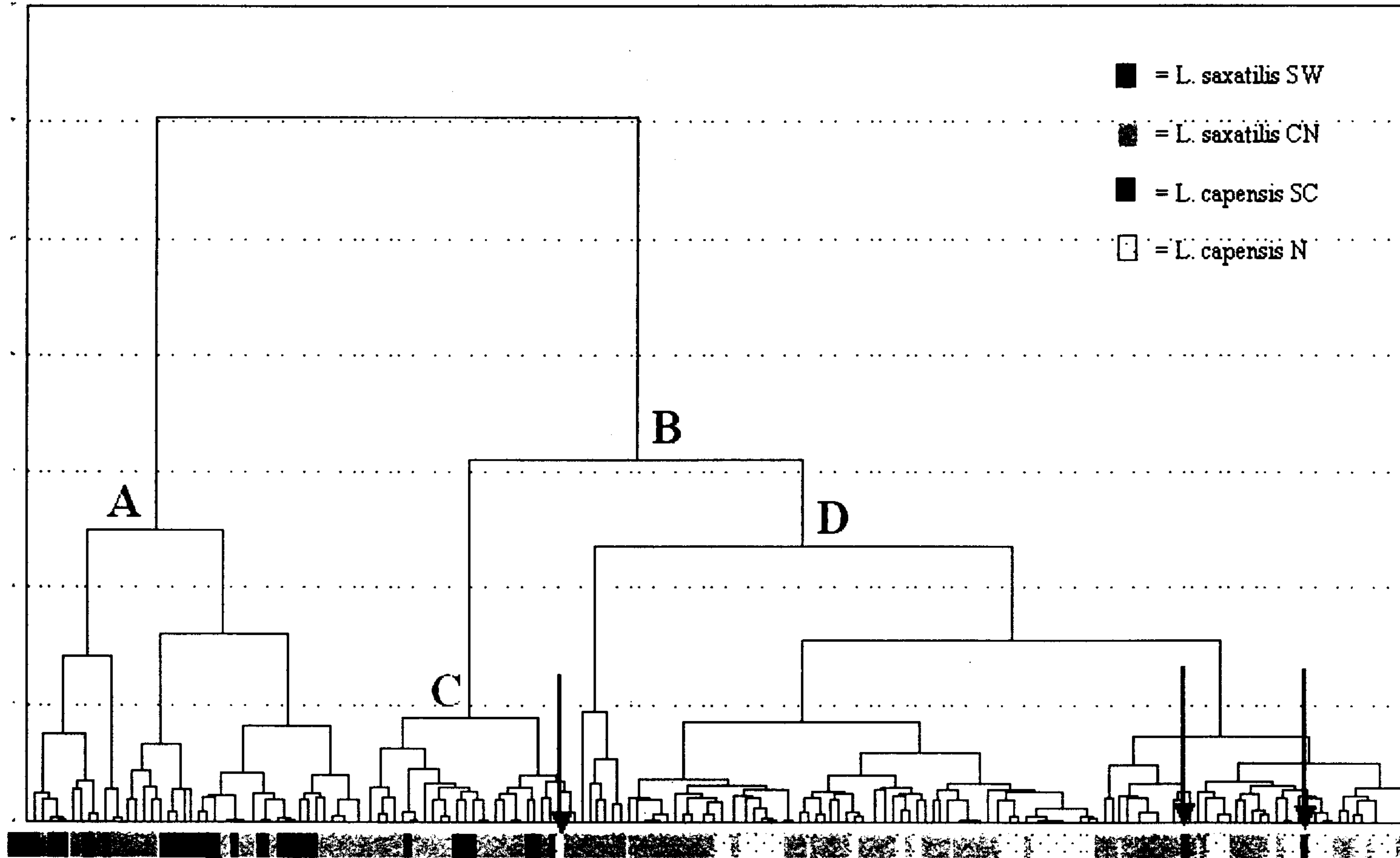

Fig. 7.5 Distance phenogram from a UPGMA cluster analysis of 177 *Lepus* specimens from southern Africa. The four hare groups correspond to the four genetically defined lineages. The arrows highlight the three individuals of uncertain placement.

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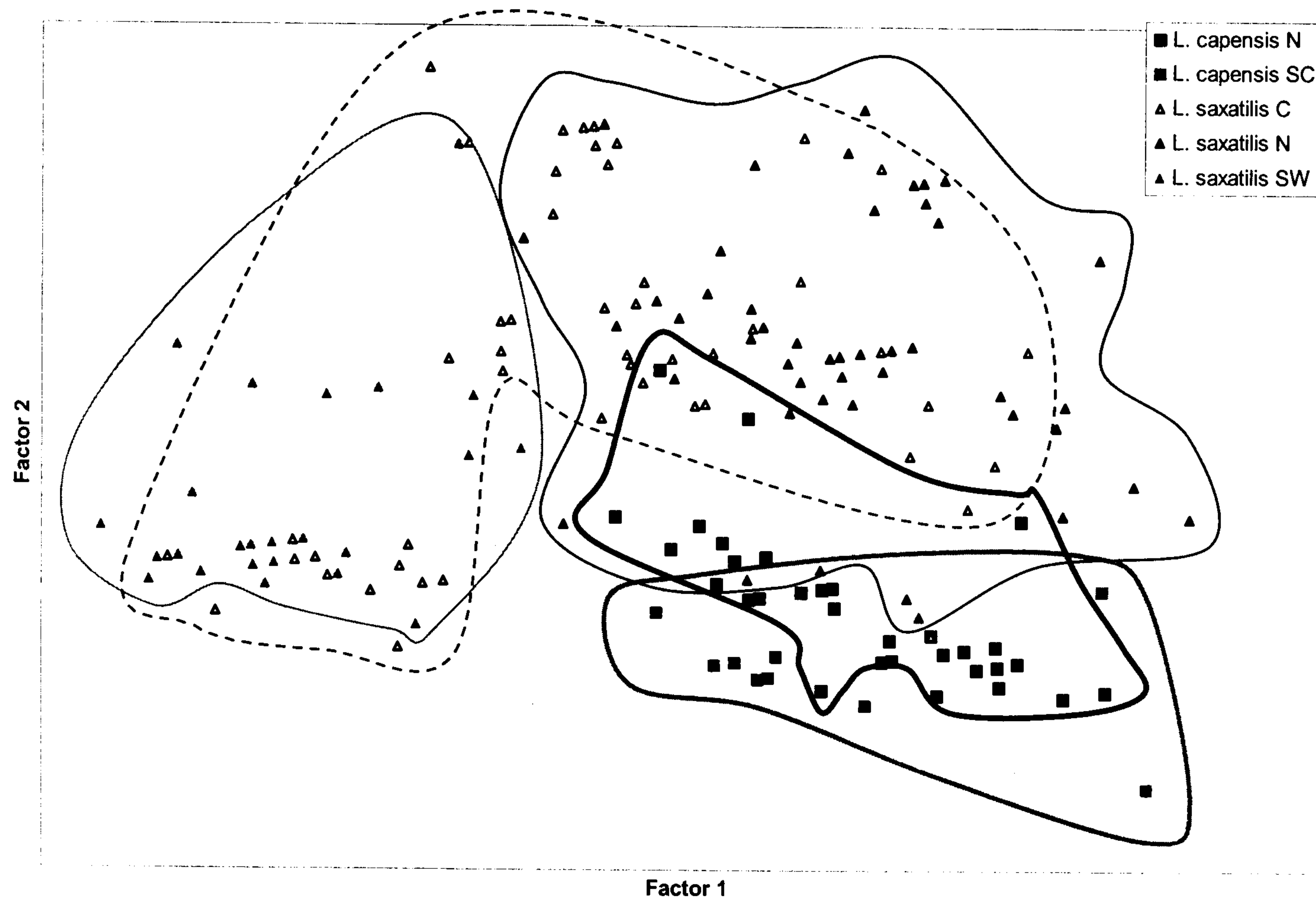

Fig. 7.6 Factors 1 and 2 from a principal component analysis of 177 *Lepus* specimens from southern Africa. The different lines circumscribe the range of the five different groups; thick lines code for *L. capensis s. l.* (gray = SC group, black = N group), thin lines for *L. saxatilis s. l.* (gray = SW group, black = N group, black interrupted = group C).

7.5 Discussion

Molecular phylogeny of South African hares

The results of the mtDNA analyses suggested that South African hares are divided into two major monophyletic lineages (Figs. 2 and 3). One comprises animals referable to *L. capensis s. l.* and the other to *L. saxatilis s. l.*. Each of these two lineages is further subdivided into two reciprocally monophyletic subclades that are geographically isolated from each other, one subclade is restricted to the south of the country and the other to the north. In both major clades a third, highly divergent lineage was discovered in addition to the two subclades. Unfortunately, each divergent mtDNA haplotype was represented by a single specimen only and increased sample sizes are clearly necessary to resolve their evolutionary relationships to other southern African *Lepus*.

Despite the conflicting evidence retrieved with the neighbor-joining analysis of the microsatellite data (Fig. 7.4) and the Fisher's exact tests (Table 7.4), the partitioning of the nuclear genetic variation as reflected in F_{ST} and R_{ST} values (Table 7.5) clearly confirmed the four mitochondrially defined clades as separate units. The results of the assignment tests (Tables 7.6 and 7.7) further corroborated this division. In previous chapters it has been pointed out that phylogenetic reconstruction based on microsatellites requires a large number of loci (50–100) in order to recover the correct topology (Takezaki & Nei 1996). Here we only applied six loci, which may explain the weak bootstrap support values and the aberrant topology. The reliability of the results of Fisher's exact tests for pairwise genetic differentiation seems limited for our data set, since three out of four comparisons between *L. saxatilis s. l.* and *L. capensis s. l.* were highly ambiguous although there is overwhelming support for recognizing these groups as separate species (LsSW-LcSC, LsCN–LcN, LsCN–LcSC; Table 7.4). For these reasons we put more confidence in the outcomes of the F_{ST}/R_{ST} calculations and assignment tests.

Dating of divergences

A molecular clock based on the assumption of a constant mutation rate through time can provide a useful guide for determining divergence times among species from their genetic sequence divergence (e.g. Pierpaoli *et al.* 1999; Pook *et al.* 2000; Yu *et al.* 2000). However, the patterns of mtDNA sequence evolution are still only poorly understood (Tamura & Nei 1993) and thus the results have to be interpreted with caution. Martin & Palumbi (1993) pointed out that nucleotide substitutions occur at different rates in different mammalian lineages and that it is impossible to assume a global molecular clock. Among closely related taxonomic groups, though, clock assumptions are considered to be more reliable (Caccone *et al.* 1997) and it is thought to be possible to estimate "local" rates (Hillis *et al.* 1996b).

In this study, the cytochrome *b* data set was characterized by a constant mutation rate over all lineages in these closely related species largely justifying the application of a molecular clock. The standard mammal calibration for cytochrome *b* is 2% divergence between two sequences per million years (Brown *et al.* 1979; Martin & Palumbi 1993; Klicka & Zink 1997). This calibration would lead to divergence dates for the basal split between the European species and the African species (19.37% pairwise sequence divergence) at 9.68 million years ago (Ma; in the mid-Miocene). The split between the closely related mountain hare and the Italian hare (3.75%) is estimated to have occurred at 1.88 Ma. The diversification of leporids is thought to have taken place much later (in the Pliocene/Pleistocene; Angermann 1973; Chapman & Flux 1990; Suchentrunk & Cervantes 1998). The divergence between the two European sister species *L. corsicanus* and *L. timidus* has been dated at 0.8 Ma (Pierpaoli *et al.* 1999). Rodents, the sister taxon to the Lagomorpha in the superorder Glires, are reported to have a higher rate of DNA sequence evolution (Wu & Li 1985; Li & Tanimura 1987), possibly due to their higher metabolic rate (Martin & Palumbi 1993). Accepting the superorder Glires as a valid taxonomic unit, it is not unlikely that lagomorphs too may similarly exhibit an accelerated rate of molecular evolution. We therefore applied the faster clock calibration of 4% sequence divergence per million years used on European hare species by Pierpaoli *et al.* (1999). Using this calibration, the split between the European and African species

included here occurred 4.84 Ma (Fig. 7.3) in the early Pliocene. This date lies well within the relevant timeframe given by Angermann (1973) and Chapman & Flux (1990) based on morphological data and confirms closely to the suggested 5 Ma inferred from molecular data (Perez-Suarez *et al.* 1994). Using this calibration, the separation between the Italian and the mountain hare dates back to 0.94 Ma (Fig. 7.3), an estimate that agrees well with Pierpaoli *et al.* (1999; 0.8 Ma); the slight discrepancy probably arose due to the differences in the number of included sequences and the distance correction used in both studies. The faster calibration suggested that the two major African clades ("capensis" and "saxatilis"), which were separated by an average of 13.8% sequence divergence, originated in the middle Pliocene 3.45 Ma (Fig. 7.3). Furthermore, the split between the southwestern and northern scrub hares was dated at the early Pleistocene 1.09 Ma, and between the south-central and northern Cape hares at 1.45 Ma (Fig. 7.3). These dates all correspond with a period of climate change that caused major shifts in African habitats that are thought to have fostered allopatric isolation and subsequent speciation in many African mammals (De Menocal 1995).

Morphological evidence

Our analyses of the few morphological characters previously considered as useful (earlength, breadth of the first upper incisor, shape of the enamel fold of the principal upper incisor, and the presence of a white spot on the forehead) showed that their taxonomic information is limited. It was not even possible to unambiguously demarcate *L. saxatilis s. l.* from *L. capensis s. l.* based on any of the four documented characters.

However, the results corroborated the findings of Robinson (1981, 1986) that a complex shape of the enamel fold of the principal upper incisor allows the unequivocal allocation of a specimen to *L. saxatilis s. l.*. Also, that the complexity of the enamel groove tends to increase from a simple shape in the southwestern scrub hares towards an increasingly complex invagination (also with higher incidence) in the northeastern scrub hares. The average of the breadth of the principal upper incisor measured in this study closely conformed to the values given by Robinson (1986). Conversely, the observed range of this character was broader (especially towards smaller values) in this study than

in Robinson (1982, 1986). A possible explanation may be that the values obtained by Robinson (1982, 1986) were taken from adult animals only, whereas this study did not assess the age and developmental stage of the included specimens.

The data are also consistent with Kingdon's observation (1974), that the presence of a white marking on the forehead is recorded more frequently in *L. saxatilis s. l.* (corresponding to Kingdon's *L. crawshayi*) than in *L. capensis s. l.* (Appendix Table A1). Nonetheless, its presence in all four hare lineages precludes its use as a diagnostic feature.

The length of the ears (along with general body size; data not shown) showed a broader range in the "saxatilis" cluster than in the "capensis" cluster and seemed to follow the described clinal decrease from southwest to northeast (Robinson & Dippenaar 1983, 1987; Skinner & Smithers 1990). We did not find evidence in support of a distinct discontinuity in ear length noted by Robinson (1981) where animals from the former provinces Natal and central southern Transvaal and Winburg in the Orange Free State had significantly shorter ears than specimens from all other localities tested in that study. However, we detected a steep decrease in scrub hare ear length (and body size; data not shown) separating a larger form in the Western Cape Province (and Loxton) from the rest of the sample localities. This break was further northeast than the genetic break separating *L saxatilis s. str.* from the central-northern lineage, but further southwest than the break suggested by Petter (1972) separating *L. saxatilis* (ears longer than 130 mm) from *L. crawshayi* (ears shorter than 130 mm). Schneider (1990) explained variations in ear length and body size in lagomorphs as being associated with habitat: following Allen's rule individuals in cold regions show smaller appendages than individuals in hot arid regions. Furthermore, it has been observed that translocated populations of *Lepus* have differentiated in accordance with Bergmann's rule in body size within 100 years and in coloration within 40 years (Schneider 1990). Roberts (1951) suggested that local differences in fur color or body size in South African mammals are common and usually correlate with differences in climate. He specifically noted a darkening of fur color in animals from the moist southern strip of the Cape Province (as compared to related forms from the dry areas further north which show less contrast in their fur) and the trend for longer ears in animals from the west of the country. This explanation would also account

for the longer ears and the dark red fur distinguishing the south-central Cape hares from the northern lineage.

The cluster analysis proved that the four morphological characters in combination could distinguish among the southwestern scrub hare lineage and the two distinct Cape hare lineages. It was not possible, though, to separate the central-northern scrub hare clade from any of the other three groups. The PCA revealed that the northern component of the central-northern scrub hare lineage is clearly distinct from the southwestern form. This separation coincided with the genetic division into subpopulations within the central-northern scrub hare (see chapters 4 and 5). The PCA identified three mutually exclusive units (the southwestern and the northern scrub hares and the south-central Cape hare) and two units demonstrating an overlap of morphological variation (the northern Cape hare and the central scrub hare). The prominent lack of a clear separation between the two recognized species (*L. saxatilis s. l.* and *L. capensis s. l.*) seems to demonstrate the influence of ecological and environmental rather than genetic factors on the morphological characters used here. Similar morphological features may simply be the consequence of similar selection pressures and would thus be convergent.

Taxonomic implications

Based on the reciprocal monophyly in two separate mitochondrial gene trees in combination with the demonstrated difference in allele frequencies at nuclear loci it can be concluded that there are at least four distinct evolutionarily significant units *sensu* Moritz (ESUs, Moritz 1994a, 1995) present within South African hares: the southwestern and the central-northern scrub hare and the south-central and the northern Cape hare. The average pairwise cytochrome *b* sequence divergence between the two scrub hare ESUs (4.4%) and between the two Cape hare ESUs (5.8%) was close to the suggested median mitochondrial sequence divergence between mammalian sister species (6.4%; Avise *et al.* 1998) and within the range of mean cytochrome *b* genetic distances reported for pairs of sister species in mammals (3–6%; Johns & Avise 1998). Furthermore, lagomorph specific cytochrome *b* sequence divergences between sister species have been reported with 3.2% between the pika species *Ochotona curzoniae* and *O. nubrica* and 3.8%

between *O. macrotis* and *O. roylei* (Yu *et al.* 2000); 3.2% between the American hare species *L. alleni* and *L. californicus* (Halanych *et al.* 1999); 4.9% between the Arctic hare species *L. othus* and *L. townsendii* (Halanych *et al.* 1999); and 3.75% between the European hare species *L. timidus* and *L. corsicanus* (Pierpaoli *et al.* 1999). In this light, the recovered sequence divergences between the two scrub hare ESUs and the two Cape hare ESUs may well indicate that *L. saxatilis s. l.* and *L. capensis s. l.* may actually represent two species groups consisting of at least two sister species each (the presence of the highly divergent specimens Z07 and X01 raised the possibility of a third species in each group).

The biological species concept does not seem adequate for the genus *Lepus*, since even distantly related species have been reported to interbreed with fertile offspring (Flux 1983; Thulin *et al.* 1997) and the mechanisms of isolation are thought to be of a geographic, behavioral, or ecological nature rather than reproductive isolation (Halanych *et al.* 1999). Adopting either the phylogenetic (Cracraft 1983, 1989) or the evolutionary species concept (Simpson 1961), all four South African hare ESUs would have to be addressed as distinct species, since they represent the smallest diagnosable clusters and distinct monophyletic evolutionary lineages. In this case, the name *L. saxatilis* F. Cuvier 1823 (*sensu stricto*) would have to be allocated to the southwestern scrub hare and the name *L. capensis* Linnaeus 1758 (*sensu stricto*) to the south-central Cape hare, due to the type localities of both species descriptions being situated in the Cape of Good Hope. The distribution of the thus newly defined *L. saxatilis s. str.* would be confined to the southwestern corner of South Africa and therefore agree with Angermann (1973, 1983). It is conceivable that the northern scrub hare represents the savanna hare (*L. victoriae sensu* Flux & Angermann 1990). However, the correct name and precise distribution limits for this northern "saxatilis" form (as well as for the northern "capensis" form) can only be assessed with more extensive geographic sampling and genetic and morphological comparisons with material previously defined as *L. victoriae*, *L. whytei*, or *L. crawshayi*.

CONCLUSIONS

Hares are important prey species in many ecosystems and therefore it is of high priority to conserve them. A precise knowledge of inter- and intraspecific taxonomic entities and their geographic distribution is a precondition for their effective conservation. The morphology based taxonomy for hares is very controversial, due to the fact that intraspecific variability of morphological characters generally exceeds interspecific variation. In this thesis I have undertaken to elucidate the boundaries among South African hares applying molecular genetic techniques.

Through sequencing of mtDNA fragments and a survey of the size variation in six microsatellite loci, four genetically discrete lineages were identified among South African hares. Due to the levels of genetic differentiation and the phylogenetic relationships among these four lineages and based on the phylogenetic species concept, the data suggested a taxonomic division of South African hares into two species groups consisting of two sibling species each: (i) *Lepus saxatilis sensu lato* encompassing *L. saxatilis sensu stricto* (restricted to the southwestem comer of the Western Cape Province) and *L. victoriae* (?; occurring throughout the central and northem regions of South Africa and extending into Namibia, Botswana, and Zimbabwe) and (ii) *L. capensis s. l.* comprising *L. capensis s. str.* (restricted to the south-central areas of South Africa) and a taxonomically yet to be identified species (distributed throughout the northem parts of South Africa and reaching into Namibia). There was no genetic evidence in support of the current subspecies delimitations in either the scrub hare or the Cape hare. In future studies, these hypotheses should be tested with additional evidence from ecology, ethology, and geometric morphometrics. Furthermore, genetic analyses should be continued including hares from the central and northern African countries (especially specimens that have been identified as *L. crawshayi*, *L. whytei*, and *L. victoriae*), in order to assess the precise taxonomical and distributional borders of hares for the entire continent. The finding of two genetically different lineages in South African *L. capensis s. l.* casts serious doubt on the validity of the currently presumed

widespread distribution of this species; a cladistic analysis of the genetic variation among Cape hares collected throughout their geographical range would clarify this issue. Future genetic analyses should ideally include more nuclear markers (either more microsatellite loci or markers such as introns), loci of adaptive significance (such as MHC), and possibly y-chromosome linked markers (in order to establish the degree of male-biased dispersal).

This study demonstrated that the four South African hare lineages originated from a common ancestor in the Pliocene and since experienced separate demographic histories and carry different evolutionary potential. Therefore they should be preserved as distinct conservation units. Special attention should be given to the conservation of the southwestern scrub hare, since its geographic distribution is limited to a small area. The data detected an indication of the presence of two additional hare lineages in southern Africa; this possibility should be investigated with more extensive sampling, especially around the areas of Bethanie (Namibia) and Acornhoek (South Africa).

Population history analyses revealed that South African hare populations underwent massive population size increases and accompanying range expansions in the Pleistocene, probably in concert with climate induced habitat changes. The phylogeographic breaks detected in *L. saxatilis s. l.* and *L. capensis s. l.* do not coincide precisely with discontinuities described for other South African small mammals. However, the comparison indicated that the Limpopo river, as well as the transition zone between the South African Highveld (grassland) and Lowveld (savanna), may have represented barriers to dispersal for various South African small mammal species.

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APPENDIX

Table A1 Sample index showing the map co-ordinates of sampling localities as derived from Skead's Zoo Historical Gazetteer (Skead 1973). Where the samples were actually obtained on farms the nearest town is mentioned first with the name of the farm in parentheses. The coding of the individuals is used throughout this thesis. The first upper incisor was measured for its breadth and was checked for the shape of the enamel groove in its anterior surface. Ear length was measured and the absence or presence of a white spot on the forehead of the animals was documented.

Locality	GPS co-ordinates	Field No	Code	Incisor Breadth	Incisor Groove	Ear length	White Spot
Lepus saxatilis s. l.							
Porterville (Farm: Grootfontein)	32°54'S 19°06'E	Po1	A01	-	-	128	
		Po2	A02	2.9	simple	135	
		Po3	A02	3.1	simple	138	
		Po4	A04	3.0	simple	140	
		Po5	A05	3.0	simple	137	present
		Po6	A06	3.1	simple	138	
		Po7	A07	2.6	simple	137	
Swellendam	34°02'S 20°26'E	Sw1	B01	3.1	simple	141	present
		Sw2	B02	3.1	complex	128	present
		Sw3	B03	2.6	simple	131	present
		Sw4	B04	3.0	simple	132	present
		Sw5	B05	2.9	simple	126	present
		Sw6	B06	3.4	simple	133	
		Sw7	B07	3.1	simple	132	
		Sw8	B08	2.9	simple	crippled	present
		Sw9	B09	2.6	simple	136	present
		Sw10	B10	3.0	simple	131	
Calvinia (Farm: Droekloof)	31°55'S 20°05'E	Calv1	C01	3.1	simple	137	
		Calv2	C02	3.1	simple	135	
		Calv3	C03	3.3	simple	145	
		Calv4	C04	3.0	simple	145	
		Calv5	C05	3.1	complex	151	
		Calv6	C06	3.0	simple	138	
		Calv7	C07	2.9	simple	142	
		Calv8	C08	3.1	simple	144	
		Calv9	C09	3.1	simple	146	
		Calv10	C10	3.0	simple	150	
Oudtshoorn (Farm: Brakrivier)	33°46'S 22°31'E	Oudt1	D01	3.0	simple	134	
		Oudt2	D02	3.0	simple	134	
		Oudt3	D03	2.8	simple	135	
		Oudt4	D04	3.0	simple	136	
		Oudt5	D05	3.0	simple	125	
		Oudt6	D06	3.1	simple	133	
		Oudt7	D07	2.8	simple	130	
		Oudt8	D08	2.9	simple	129	
		Oudt9	D09	3.1	simple	133	
		Oudt10	D10	2.9	simple	136	
Loxton (Farm: Rietpoort)	31°38'S 22°22'E	Lox1	E01	-	-	-	-
		Lox2	E02	2.8	simple	150	
		Lox3	E03	2.5	simple	142	
		Lox4	E04	3.1	simple	145	
		Lox5	E05	3.0	simple	138	
Grahamstown	33°30'S 26°29'E	Grah1	F01	2.7	simple	110	present
		Grah2	F02	3.1	complex	118	present
		Grah3	F03	3.0	complex	123	
		Grah4	F04	2.0	simple	107	present

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Locality	GPS co-ordinates	Field No	Abbrev.	Incisor Breadth	Incisor Groove	Ear length	White Spot
Grahamstown	33°30'S 26°08'E	Grah5	F05	3.0	complex	110	
		Grah6	F06	3.0	complex	113	
		Grah7	F07	3.0	complex	118	present
		Grah8	F08	3.1	simple	117	present
		Grah9	F09	3.1	simple (deep)	122	present
		Grah10	F10	3.0	simple (deep)	108	present
Kirkwood	33°24'S 25°25'E	Kirkw	F11	2.8	simple	128	
Tussen-die-Riviere GR	30°28'S 26°12'E	TdR1	G01	3.5	complex	118	present
		TdR2	G02	3.1	complex	115	present
		TdR3	G03	3.0	complex	122	
		TdR4	G04	2.9	complex	125	present
		TdR5	G05	3.1	complex	127	present
		TdR6	G06	2.7	simple	120	present
		TdR7	G07	3.5	miss	128	
		TdR8	G08	3.0	complex	116	present
		TdR9	G09	2.9	complex	120	present
		TdR10	G10	3.0	simple	120	present
Port Shepstone	30°45'S 30°27'E	SKZN1	H01	3.1	complex	106	
		SKZN2	H02	2.2	simple	100	
Harding	30°34'S 29°52'E	SKZN3	H03	-	-	-	
Kimberley	28°45'S 24°45'E	Kimb6	J06	3.0	complex	110	
		Kimb7	J07	2.6	complex	122	
		Kimb10	J10	2.7	complex	120	
Willem-Pretorius GR	28°18'S 27°15'E	WPret1	K01	2.4	complex	111	
		WPret2	K02	2.7	complex	112	
		WPret3	K03	2.5	complex	110	
		WPret4	K04	3.4	complex	108	
		WPret5	K05	2.9	complex	109	
		WPret6	K06	2.7	simple	111	present
		WPret7	K07	2.6	complex	119	
		WPret8	K08	2.5	complex	107	present
		WPret9	K09	2.6	complex	104	
		WPret10	K10	3.1	complex	114	present
Vryheid	27°46'S 30°48'E	Vryh1 / JVN1	L01	3.0	complex	110	
		Vryh2 / JVN2	L02	2.6	simple	99	present
		Vryh3 / JVN4	L03	2.7	complex	116	
		Vryh4 / WB1	L04	2.3	simple	103	present
		Vryh5 / WB2	L05	3.1	complex	116	present
		Vryh6 / WB3	L06	3.5	complex	102	
		Vryh7 / TR1	L07	2.6	complex	101	
		Vryh8 / TR2	L08	2.7	complex	100	present
		Vryh9 / TR3	L09	2.0	simple	-	present
Ermelo	26°31'S 29°59'E	Erme4 / Nolte1	P04	2.4	complex	-	
Amsterdam	26°36'S 30°39'E	Erme13 / DG5	P13	2.6	complex	100	present
Vryburg	26°57'S 24°44'E	Vryb	S01	1.9	simple	101	present

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Locality	GPS co-ordinates		Field No	Abbr ev.	Incisor Breadth	Incisor Groove	Ear length	White Spot
Rustenburg NR	25°40'S	27°15'E	Rust1	T01	2.9	complex	101	present
			Rust2	T02	2.1	complex	98	present
			Rust3	T03	3.0	simple	96	present
Pilanesberg NP	25°12'S	27°05'E	Pila1	T04	2.9	complex	115	
			Pila2	T05	3.0	-	106	
			Pila3	T06	2.6	complex	103	
			Pila4	T07	2.8	complex	100	present
			Pila5	T08	2.6	complex	108	
			Pila6	T09	2.8	simple	106	
			Pila7	T10	2.2	simple	98	present
			Pila8	T11	3.0	complex	108	
			Pila9	T12	2.6	simple	105	
			Pila10	T13	2.3	complex	104	
Pretoria	25°45'S	28°10'E	PTA1	U01	-	-	-	-
			PTA2	U02	-	-	-	-
			PTA3	U03	-	-	-	-
			PTA4 / Clarke	U04	-	-	-	-
			PTA5 / Lisel	U05	2.2	complex	99	
			PTA6 / Martin	U06	-	-	-	-
			PTA7 / Tyron	U07	2.6	simple	98	
Pienaars Rivier Airforce NatureRes.	25°12'S	28°17'E	PiRiv1	U08	2.5	complex	110	
			PiRiv2	U09	2.9	complex	102	
			PiRiv3	U10	2.6	complex	96	-
			PiRiv4	U11	2.5	complex	106	
			PiRiv5	U12	3.0	simple	110	
			PiRiv6	U13	-	-	108	
			PiRiv7	U14	2.8	complex	109	present
			PiRiv8	U15	2.5	complex	102	
			PiRiv9	U16	2.3	simple	98	
			PiRiv10	U17	2.4	complex	104	present
Vaalwater	24°10'S	28°06'E	Vaal1 / CS1	V01	2.7	complex	104	
			Vaal2 / CS2	V02	2.5	complex	102	
			Vaal3 / CS3	V03	2.0	simple	91	present
Thabazimbi	24°36'S	27°24'E	Thaba1	V04	3.0	complex	92	present
			Thaba2	V05	2.6	complex	97	present
			Thaba3	V06	2.6	complex	98	
Hoedspruit Airforce Nature Reserve	24°22'S	30°57'E	Hoed1	W01	3.0	complex	94	
			Hoed2	W02	2.8	complex	105	
			Hoed3	W03	2.5	complex	88	
			Hoed4	W04	2.6	complex	99	
			Hoed5	W05	2.4	simple	96	
			Hoed6	W06	2.1	complex	98	
Messina (Farm: Samaria)	22°12'S	29°17'E	Mess1	Y01	2.8	complex	104	
			Mess2	Y02	2.6	complex	100	present
			Mess3	Y03	2.2	complex	94	
			Mess4	Y04	2.3	complex	97	
			Mess5	Y05	3.0	complex	103	
			Mess6	Y06	2.6	complex	97	present
			Mess7	Y07	2.6	complex	99	present
			Mess8	Y08	2.6	complex	101	
			Mess9	Y09	2.5	complex	102	present

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Otavi / Namibia	19°40'S	17°24'E	Nami1 / WD1	Z01	2.6	simple	105	present
Otavi / Namibia			Nami2 / WD2	Z02	1.8	simple	92	present
Namibia	26°08'S	17°1.9'E	Nami3 / Nig1	Z03	-	-	-	
Maltahoehe / Namibia	24°45'S	17°02'E	Nami4 / KC	Z04	2.4	complex	125	present
Bethanie / Namibia	26°32'S	17°11'E	Nami7	Z07	-	-	-	
Ghanzi / Botswana	21°50'S	21°45'E	Ghanzi	Gha	-	-	-	-
Bulawayo / Zimbabwe	20°07'S	28°32'E	Zim1	Zi01	-	-	-	-
			Zim2	Zi02	-	-	-	-
			Zim3	Zi03	-	-	-	-
			Zim4	Zi04	-	-	-	-
			Zim5	Zi05	-	-	-	-
			Zim6	Zi06	-	-	-	-
			Zim7	Zi07	-	-	-	-
			Zim8	Zi08	-	-	-	-
			Zim9	Zi09	-	-	-	-
			Zim10	Zi10	-	-	-	-
Lepus capensis s. l.					-	-	-	-
Upington	28°27'S	21°15'E	Upi	I01	-	-	-	-
Kimberley (Farm: Secretaris)	28°43'S	24°30'E	Kim1	J01	1.9	simple	105	present
			Kim2	J02	2.5	simple	100	
			Kim3	J03	2.1	simple	100	
			Kim4	J04	2.0	simple	102	
			Kim5	J05	2.6	simple	108	
Kimberley	28°45'S	24°45'E	Kim8	J08	2.2	simple	100	
			Kim9	J09	2.5	simple	108	
Hoopstad	27°50'S	25°55'E	Hoop1	M01	-	-	-	-
			Hoop2	M02	-	-	-	-
			Hoop3	M03	-	-	-	-
Fochville (Farm: Taaiboschspruit)	26°51'S	30°21'E	Sny / Pot	N01	2.8	simple	106	
Parys	26°53'S	27°35'E	Parys1	O01	2.1	simple	97	
			Parys2	O02	2.8	simple	101	
			Parys3	O03	2.5	simple	103	
			Parys4	O04	2.1	simple	100	
			Parys5	O05	2.1	simple	102	
			Parys6	O06	2.8	simple	106	
			Parys7	O07	2.0	simple	100	
			Parys8	O08	1.8	simple	96	
			Parys9	O09	2.0	-	92	
			Parys10	O10	2.5	simple	101	
Standerton	26°57'S	29°15'E	Erme1	P01	2.7	simple	-	
			Erme2	P02	3.0	simple	105	
			Erme3	P03	2.9	simple	100	
Ermelo	26°31'S	29°59'E	Erme5 / Nolte2	P05	2.1	simple	-	
			Erme6 / Nolte3	P06	2.7	simple	103	
			Erme7 / Nolte4	P07	2.1	simple	100	
			Erme8 / Nolte5	P08	2.7	simple	100	
Amsterdam	26°36'S	30°39'E	Erme9 / DG1	P09	2.9	simple	108	present
			Erme10 / DG2	P10	3.0	miss	104	

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Locality	GPS co-ordinates		Field No	Abbr ev.	Incisor Breadth	Incisor Groove	Ear length	White Spot
Amsterdam	26°36'S	30°39'E	Erme12 / DG4	P12	2.8	simple	106	
			DG6	P14	2.5	simple	107	
Kalahari	26°36'S	20°15'E	Kala1	Q01	1.8	simple	100	
			Kala2	Q02	2.0	simple	117	
			Kala3	Q03	2.1	simple	107	
			Kala4	Q04	1.9	simple	103	
			Kala5	Q05	2.0	simple	98	
			Kala6	Q06	2.1	simple	108	
			Kala7	Q07	2.2	simple	104	
			Kala8	Q08	2.1	simple	107	
			Kala9	Q09	1.9	simple	109	
			Kala10	Q10	2.2	simple	104	
Hotazel	27°12'S	23°01'E	Hot	R01	2.4	simple	103	
Acornhoek	24°36'S	31°04'E	KNP	X01	-	-	-	-
Namibia	26°14'S	17°02'E	Nami5 / Nig2	Z05	-	-	-	-
Namibia	26°24'S	17°06'E	Nami6 / Nig3	Z06	-	-	-	-
Namibia – Helmeringhausen	25°53'S	16°50'E	Nami8	Z08	-	-	-	-
Namibia	26°37'S	16°05'E	Nami9	Z09	-	-	-	-
Victoria West	31°24'S	23°07'E		CV1	2.2	simple	119	
				CV2	2.2	simple	121	
				CV3	1.5	simple	110	present
				CV4	1.9	simple	116	
				CV5	2.1	simple	119	
				CV6	2.5	simple	117	
				CV7	2.5	simple	131	present
				CV8	1.3	simple	108	
				CV9	2.2	simple	115	
				CV10	2.1	simple	120	

Table A2 Alignment of a 420 bp long fragment of the mtDNA CR-I of South African hares. Coding of individuals as in Table A1.

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A01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
A07   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
B01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-TCTTTA-TGCTAT
B04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B07   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B08   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B09   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
B10   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
C01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C07   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C08   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C09   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
C10   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
D01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--TCTTTA-TGCTAT
D06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D07   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D08   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D09   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
D10   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
E01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
E02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
E03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
E04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
E05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
F03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
F05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
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F08   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F09   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F10   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
F11   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
G01   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
G02   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
G03   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
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G05   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
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G07   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
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G10   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTA-TGCTAT
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J02   TCCTAATGAACACGTCAAAGTATCTCATCACTATTGACAAAAA-TCCATTTTAACACTAT
J03   TCCTAATGAACACGTCAAAGTATCTCATCACTATTGACAAAAA-TCCATTTTAACACTAT
J04   TCCTAATGAACACGTCAAAGTATCTCGTCACTATTGACAAAAA-TCCATTTTAACGCTAT
J05   TCCTAATGAACACGTCAAAGTATCTCATCACTATTGACAAAAA-TCCATTTTAACACTAT
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L04   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTA-TGCTAT
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L06   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
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V06   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
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W06   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
X01   TCCTAATAAACACGTCAAAGTACCTTGTCACTATTGACAAAAA-TTCATTT-AACACTAT
Y01   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Y02   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Y03   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Y04   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Y05   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
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Y07   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Y08   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
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Y10   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Z01   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Z02   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
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Z04   TCTTAATTAACATACCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Z05   TCCTAATGAACACGTCAAAGTATCTCGTCACTATTGACAAAAA-TCCATTTTAACACTAT
Z06   TCCTAATGAACACGTCAAAGTATCTCGTCACTATTGACAAAAA-TCCATTTTAACACTAT
Z07   TCTTAACTAACACATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Z08   TCCTAATGAACACGTCAAAGTATCTCGTCACTATTGACAAAAA-TCCATTTTAACACTAT
Z09   TCCTAATAAACACGTCAAAGTATCTCGTCACTATTGACAAAAA-TCCATTTTAACACTAT
Gha   TCCTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi1   TCTTAATTAACATACCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi2   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi3   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi4   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Zi5   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAAACCTTTTA-TGCTAT
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Zi7   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAA--CCTTTTA-TGCTAT
Zi8   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi9   TCTTAATTAACATACCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Zi0   TCTTAATTAACATATCCAAGTACCTTGTCACTATTGACAAAAAAA-CCTTTTA-TGCTAT
Bu1   CTACTTTTACAAAACTTAAGTGCTATGTTAGTACTGACAAAT-CATACTTCCC-CAATAT
Bu2   CTACTTTTACAAAACTTAAGTGCTATGTTAGTACTGACAAAT-CATACTTCCC-CAATAT
PRa   AACTACCCA---TATCTAAGTGCCTTGTCAGTATTGACAAAAAA-TTTTTCAA-TGCTAT
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PPa   TTTTACCCA---TATTTAAGTGCCTTGTCAGTATTGACAAAAAAATTTTTCAA-TGCTAT
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eur   TCTTAATAACATATCCAAGTAACTTGTCACTATTGACAAAA---CACCCTTTAATGCTAT
swi   TCCTAATAACAATTCCAAGTACCTTGTCACTATTGACAAAAA--TTCCCTTTAACGCTAT
man   TCCTAATAACAAATCCAAGTACCTTCTCACTATTGACAAAAA--TTCCCCTTAACGCTAT
oio   TCCTAATAACAATTCCAAGTACCTTGTCACTATTGACAAAAA--TTCCCTTTAACGCTAT
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swi   GTAATTCGTGCATTAGTGCCTTTCCCCATTAACA-TGTATCTATACTATTATCTTATAAT
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Z05   G-ACTAACAATATGACTATCCAAGTCCAACA--CTCACCTTAATCAACATCCAGACATTC
Z06   G-ACTAACAATATGACTATCCAAGTCCAACA--CTCACCTTAATCAACATCCAGACATTC
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Z08   G-ACCAACAATACGACTATCCAAGTCCAACA-CTCAATCTTAATCAACATCCAGACATTC
Z09   G-ACCAACAATACGACTATCCAAGTCCAACA--CCCACCTTAATCAACATCCAGACATTC
Gha   T-ATCAACAACATGGATATTCACACCCAACT--CTCACCTTAATCAACATCCAGACATCC
Zi1   T-ATCAACAACACGAGTATTCACATCCAATT--CTCACCTTAATCAACATCCAGACATTC
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Zi0   T-ATCAACAACACGAATATTCATATCCAATT--CTCACCTTAACCAACATCCAGACATTC
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Bu2   T-C----CAACACTACCA-------CGAATA------CCCTTATCCCCACCTAATGATTA
PPa   TGATTTCCAACACGGATATTCACCCCAAACCCACCACCCTTGATCAACATA-GCACATTA
PRa   TGACTTCCAACACGGATATTCACCCCAAACCCACCATCCTTGATCAACATA-GCACATTA
PRu   AGTCCTTCAACACGAATATTCACCTCCAAC-TCAAATCCTTAACGACCAGA-GCACATTC
CK1   G-ACCAACAATACGACTATCCAAGTCCAACA--CTCACCTTAATCAACATCCAGACATTC
CK2   G-ATCAGCAACATGACTATCCAACTCCAACA--CCCACCTTAATCAACATCCTGACATTC
CV1   T-ACCAACAACACGATTATTCAACTCCAACG--CTCACCTTAATCAACATCCAGACATAC
CV2   T-ACCAACAACACGATTATTCAACTCCAACG--CTCACCTTAATCAACATCCAGACATAC
CL1   T-ATTGACAACACGACTATTCAATTCCAATG--CTCACCTTAATCAACATCTAGACATAC
CL2   T-ATTGACAACACGACTATTCAATTCCAATG--CTCACCTTAATCAACATCTAGACATAC
eur   T-ATCACCAACATGGATATTCAATTCCAACA--CCCACCTTAATCAACATCCAGACATCC
swi   T-ACTATCAACACGAATATTAACAACCCAAT-ATCCACCTTAATCAACATTCAGACATCT
man   T-ACCACCAACACGAATACCCACAACCCAATTACCCACCTTAACCAACATCCAGACATTC
oio   T-ATCACCAACACGGATATTAACAACCCAAC-ACCCACCTTAATCAACATTCAGACATTC
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M03   A-TTCCTTAACTGGACATAAACCATCCAAGTCAAATCCTTTCTCGTCCATATGACTATCC
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S01   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T01   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T02   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T03   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T04   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T05   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T06   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T07   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T08   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
T09   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
T10   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
T11   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
T12   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
T13   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U01   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
U02   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
U03   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
U04   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U05   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U06   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U07   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U08   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U09   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U10   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U11   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U12   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
U13   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U14   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
U15   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
U16   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
U17   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
V01   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
V02   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
V03   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
V04   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
V05   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
V06   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W01   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W02   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W03   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W04   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W05   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
W06   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
X01   CCCTCCC-TTAGGAGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCW
Y01   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Y02   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Y03   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
Y04   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
Y05   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
Y06   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Y07   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Y08   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Y09   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Y10   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Z01   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Z02   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
Z03   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Z04   CCCTCCC-CCAGGAGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Z05   CCTTCCC-TTAGGAATTCCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
Z06   CCTTCCC-TTAGGAATTCCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
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Z07   CCCTCCC-CCAGGAATCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Z08   CCTTCCC-TTAGGAATTTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
Z09   CCTTCCC-TTAGAAGTTTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
Gha   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCY
Zi1   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Zi2   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
Zi3   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi4   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi5   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi6   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi7   CCTTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi8   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Zi9   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
Zi0   CCCTCCC-CCAGGGGTCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
Bu1   ATTTCCC-TTATTTCTCACACAACTTACCATCCTCCGTGAAACCAGCAACCCGCCCACAT
Bu2   ATTTCCC-TTATTTCTCACACAACTTACCATCCTCCGTGAAACCAGCAACCCGCCCACAT
PPa   CCTTCCA-CCAGTGGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
PRa   CCTTCCA-CCAGTGGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
PRu   CTGTCCA-CCCGTGGTCTCTTATTCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
CK1   CCTTCCC-TTAGAAATTTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
CK2   CCTTCCC-TTAGAGGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
CV1   CTCCCCC-CTGGAAGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
CV2   CTCCCCC-CTGGAAGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
CL1   CCTTCCC-TTGGGAGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
CL2   CCTTCCC-TTGGGAGTCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCA
eur   CCCTCCC-CCAGTAATCTCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
swi   CCCTCCCTCCAGGAATCCCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCC
man   CCTTCCC-TTAGGAATCCCTTGATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACCT
oio   CCTTCCC-TTAGGAATCCCTTAATCTACCATCCTCCGTGAAACCAGCAACCCGCCCACTT
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Table A3 Pairwise (HKY85 Γ-corrected) sequence divergences based on a 420 bp long fragment of CR-I among scrub hares and a few Cape hares as outgroup. Coding of individuals as in Table A1.

	A01	A02	A04	B01	B02	B03	C01	C02	C04	C05	D01	D02	D03	D04	F02	F05	F06	F07	F10	G01	G02	G03	G04	G06
A01		7	7	3	4	4	4	4	4	7	20	20	22	22	20	20	21	19	20	21	19	22	16	20
A02	2.43%		4	7	8	8	3	3	3	6	21	20	22	22	21	21	22	20	20	22	18	20	16	18
A04	2.43%	1.57%		7	8	8	3	3	3	6	22	23	24	26	24	24	25	22	23	25	21	21	20	16
B01	1.02%	2.43%	2.43%		1	1	4	4	4	7	20	20	22	22	20	20	21	19	20	24	19	22	16	20
B02	1.29%	2.73%	2.73%	0.25%		2	4	4	4	6	21	20	22	22	21	21	22	20	20	25	20	21	15	20
B03	1.29%	2.73%	2.73%	0.25%	0.50%		4	4	4	8	21	20	22	22	21	21	22	20	20	23	20	21	16	21
C01	1.29%	1.02%	1.02%	1.29%	1.57%	1.57%		0	2	3	21	20	22	22	21	21	22	20	20	22	18	20	16	18
C02	1.29%	1.02%	1.02%	1.29%	1.57%	1.57%	0.00%		2	3	21	20	22	22	21	21	22	20	20	22	18	20	16	18
C04	1.29%	1.02%	1.02%	1.29%	1.57%	1.57%	0.50%	0.50%		4	21	20	22	22	21	21	22	20	20	22	20	20	16	18
C05	2.43%	2.14%	2.14%	2.43%	2.14%	2.73%	1.02%	1.02%	1.57%		21	20	22	22	21	21	22	20	20	22	18	18	15	16
D01	8.79%	9.24%	10.15%	8.80%	9.26%	9.25%	9.24%	9.24%	9.24%	9.26%		1	2	3	2	2	4	3	2	8	3	16	9	11
D02	8.36%	8.80%	10.63%	8.37%	8.81%	8.80%	8.80%	8.80%	8.80%	8.81%	0.25%		2	2	1	1	3	2	2	8	2	17	8	12
D03	9.66%	10.13%	11.09%	9.67%	10.15%	10.14%	10.13%	10.13%	10.13%	10.15%	0.50%	0.76%		2	3	3	5	4	4	10	4	18	10	13
D04	9.67%	10.14%	12.11%	9.68%	10.16%	10.15%	10.14%	10.14%	10.14%	10.16%	1.02%	0.76%	0.50%		3	3	5	4	4	10	4	18	9	13
F02	8.78%	9.23%	11.11%	8.79%	9.25%	9.24%	9.23%	9.23%	9.23%	9.25%	0.50%	0.25%	1.02%	1.02%		0	4	2	1	7	3	18	9	13
F05	8.78%	9.23%	11.11%	8.79%	9.25%	9.24%	9.23%	9.23%	9.23%	9.25%	0.50%	0.25%	1.02%	1.02%	0.00%		4	2	1	7	3	18	9	13
F06	9.20%	9.66%	11.57%	9.21%	9.67%	9.67%	9.66%	9.66%	9.66%	9.67%	1.29%	1.02%	1.85%	1.85%	1.29%	1.29%		5	4	9	5	19	10	14
F07	7.94%	8.37%	10.16%	7.95%	8.38%	8.37%	8.37%	8.37%	8.37%	8.38%	1.02%	0.76%	1.57%	1.57%	0.50%	0.50%	1.85%		1	8	4	16	9	13
F10	8.36%	8.80%	10.63%	8.37%	8.81%	8.80%	8.80%	8.80%	8.80%	8.81%	0.76%	0.50%	1.29%	1.29%	0.25%	0.25%	1.57%	0.25%		8	4	17	10	14
G01	9.22%	9.67%	11.59%	11.11%	11.62%	10.62%	9.67%	9.67%	9.67%	9.69%	3.04%	2.73%	3.67%	3.67%	2.43%	2.43%	3.35%	3.04%	2.73%		10	17	13	14
G02	7.95%	7.53%	9.24%	7.95%	8.39%	8.38%	7.53%	7.53%	8.37%	7.55%	1.02%	0.76%	1.57%	1.57%	1.02%	1.02%	1.85%	1.57%	1.29%	3.67%		16	10	14
G03	9.75%	8.42%	9.29%	9.76%	9.30%	9.31%	8.42%	8.42%	8.42%	7.59%	6.77%	7.16%	7.57%	7.58%	7.57%	7.57%	7.99%	6.77%	7.16%	7.16%	6.77%		17	16
G04	6.35%	6.74%	8.37%	6.36%	5.99%	6.74%	6.74%	6.74%	6.74%	5.99%	3.36%	3.04%	4.00%	3.35%	3.35%	3.35%	3.67%	3.36%	3.68%	5.03%	4.01%	7.16%		5
G06	8.79%	7.53%	6.73%	8.80%	8.38%	9.24%	7.53%	7.53%	7.53%	6.74%	4.34%	4.68%	5.03%	5.03%	5.03%	5.03%	5.38%	5.04%	5.40%	5.39%	5.77%	6.76%	1.85%	
G10	10.11%	10.58%	12.59%	12.08%	12.63%	11.58%	10.58%	10.58%	10.58%	10.60%	3.67%	3.35%	4.32%	4.32%	3.03%	3.03%	3.99%	3.67%	3.35%	0.50%	4.33%	7.98%	5.75%	6.12%
H01	7.54%	7.95%	7.95%	7.54%	7.15%	7.96%	6.36%	6.36%	6.36%	4.92%	5.04%	4.69%	5.76%	5.03%	5.04%	5.04%	5.39%	5.04%	5.40%	5.39%	5.78%	7.57%	1.85%	2.14%
J06	9.67%	10.13%	12.10%	11.60%	12.14%	11.11%	10.13%	10.13%	10.13%	10.15%	3.35%	3.04%	3.99%	4.00%	2.73%	2.73%	3.67%	3.35%	3.04%	0.25%	4.00%	6.77%	5.39%	5.76%
J07	8.78%	9.23%	11.11%	8.79%	9.25%	9.24%	9.23%	9.23%	9.23%	9.25%	0.50%	0.25%	1.02%	1.02%	0.00%	0.00%	1.29%	0.50%	0.25%	2.43%	1.02%	7.57%	3.35%	5.03%
K01	5.59%	4.55%	4.55%	5.60%	5.96%	5.96%	4.55%	4.55%	4.55%	5.96%	6.89%	6.50%	7.68%	6.88%	6.88%	6.88%	7.27%	6.11%	6.50%	7.27%	7.70%	6.01%	6.12%	5.74%
K03	4.89%	3.90%	5.24%	4.89%	5.25%	5.24%	3.90%	3.90%	3.90%	5.25%	6.11%	5.74%	6.88%	6.11%	6.11%	6.11%	6.48%	5.38%	5.74%	6.49%	6.89%	5.29%	5.38%	6.50%
K05	4.89%	3.90%	5.24%	4.89%	5.25%	5.24%	3.90%	3.90%	3.90%	5.25%	6.11%	5.74%	6.88%	6.11%	6.11%	6.11%	6.48%	5.38%	5.74%	6.49%	6.89%	5.29%	5.38%	6.50%
K06	6.35%	5.25%	6.73%	6.35%	5.98%	6.73%	5.25%	5.25%	5.25%	4.57%	4.00%	3.67%	4.67%	4.00%	4.00%	4.00%	4.33%	4.00%	4.33%	4.33%	3.35%	5.28%	1.57%	1.85%
L01	9.75%	9.30%	10.23%	9.77%	10.25%	9.32%	9.30%	9.30%	9.30%	9.30%	3.37%	3.69%	4.02%	4.03%	4.02%	4.02%	4.36%	3.37%	3.69%	4.37%	4.71%	2.13%	3.69%	4.04%
L02	4.65%	4.31%	5.73%	4.66%	5.01%	5.01%	4.31%	4.31%	4.31%	5.73%	3.74%	3.42%	4.42%	4.43%	3.75%	3.75%	4.08%	4.43%	4.08%	4.79%	4.44%	6.09%	4.78%	6.69%
L05	6.18%	5.78%	7.38%	6.18%	5.80%	6.57%	5.78%	5.78%	5.78%	5.03%	2.19%	1.90%	2.81%	2.21%	2.20%	2.20%	2.50%	2.20%	2.50%	3.13%	2.83%	4.37%	0.00%	1.35%
L06	6.73%	5.61%	7.12%	6.73%	7.13%	7.12%	5.61%	5.61%	5.61%	5.61%	4.34%	4.00%	5.02%	4.33%	4.33%	4.33%	4.67%	4.34%	4.68%	4.67%	3.67%	6.39%	2.43%	2.73%
L09	6.72%	5.60%	7.11%	6.72%	7.12%	7.11%	5.60%	5.60%	5.60%	7.12%	6.51%	6.12%	7.29%	7.29%	6.50%	6.50%	6.88%	6.51%	6.12%	6.89%	7.31%	6.74%	7.31%	8.57%
S01	4.24%	3.28%	4.57%	4.25%	4.58%	4.58%	3.28%	3.28%	3.28%	4.58%	5.40%	5.04%	6.13%	5.39%	5.40%	5.40%	5.75%	4.69%	5.04%	5.75%	6.15%	6.04%	4.69%	5.76%
T01	8.83%	7.56%	8.40%	8.84%	8.42%	9.29%	7.56%	7.56%	7.56%	6.78%	4.36%	4.71%	5.05%	5.05%	5.06%	5.06%	5.41%	5.06%	5.42%	5.41%	5.80%	6.79%	1.86%	1.03%
T02	3.59%	3.91%	5.26%	3.60%	3.92%	3.92%	3.91%	3.91%	3.91%	5.27%	4.68%	4.34%	5.38%	5.39%	4.68%	4.68%	5.02%	4.01%	4.34%	6.51%	5.40%	7.60%	4.68%	7.31%
T08	5.61%	4.57%	5.97%	5.62%	5.99%	5.99%	4.57%	4.57%	4.57%	5.99%	6.91%	6.52%	7.71%	6.90%	6.90%	6.90%	7.29%	6.14%	6.52%	7.29%	7.74%	6.03%	6.13%	7.30%
T12	4.89%	3.90%	5.24%	4.89%	5.25%	5.24%	3.90%	3.90%	3.90%	5.25%	6.11%	5.74%	6.88%	6.11%	6.11%	6.11%	6.48%	5.38%	5.74%	6.49%	6.89%	5.29%	5.38%	6.50%
U06	5.98%	4.91%	6.35%	5.99%	6.36%	6.36%	4.91%	4.91%	4.91%	6.36%	6.52%	6.13%	7.29%	6.51%	6.51%	6.51%	7.70%	5.76%	6.13%	6.12%	7.32%	6.41%	6.52%	7.72%
V03	4.23%	3.27%	4.56%	4.23%	4.56%	4.56%	3.27%	3.27%	3.27%	4.56%	5.38%	5.03%	6.11%	5.38%	5.38%	5.38%	5.74%	4.68%	5.03%	5.74%	6.13%	6.03%	4.68%	5.75%
W01	10.93%	9.54%	11.42%	10.95%	11.45%	10.47%	9.54%	9.54%	9.54%	9.55%	6.42%	6.05%	7.19%	6.42%	6.42%	6.42%	6.79%	5.68%	6.05%	6.04%	5.68%	2.42%	6.43%	6.81%
W03	11.37%	11.88%	12.91%	13.46%	14.04%	12.93%	11.88%	11.88%	11.88%	11.90%	6.03%	6.41%	6.78%	6.78%	6.79%	6.79%	7.17%	6.03%	6.41%	4.94%	7.59%	3.34%	6.79%	6.41%
W05	4.55%	3.58%	4.89%	4.55%	4.90%	4.89%	3.58%	3.58%	3.58%	4.90%	5.74%	5.37%	6.48%	6.49%	5.74%	5.74%	6.10%	5.02%	5.37%	6.10%	6.50%	5.65%	5.74%	6.89%
Y02	5.24%	4.22%	5.59%	5.24%	5.60%	5.60%	4.22%	4.22%	4.22%	5.60%	6.50%	6.11%	7.27%	6.49%	6.49%	6.49%	6.87%	5.74%	6.11%	6.88%	7.29%	5.65%	5.74%	6.89%
Y03	4.91%	5.26%	6.73%	4.92%	5.27%	5.27%	5.26%	5.26%	5.26%	6.75%	5.39%	5.03%	6.12%	6.12%	5.39%	5.39%	5.74%	5.39%	5.03%	5.74%	6.14%	7.59%	6.13%	9.02%
Y06	4.22%	4.55%	5.96%	4.22%	4.56%	4.56%	4.55%	4.55%	4.55%	5.97%	5.38%	5.02%	6.11%	5.37%	5.37%	5.37%	5.73%	4.67%	5.02%	7.28%	6.12%	6.02%	4.67%	7.29%
Y09	7.10%	5.96%	7.49%	7.10%	7.51%	7.50%	5.96%	5.96%	5.96%	7.51%	5.37%	5.02%	6.10%	5.37%	5.37%	5.37%	5.73%	4.67%	5.02%	5.73%	6.12%	6.76%	5.38%	6.50%
Y10	5.96%	4.89%	5.60%	5.96%	6.34%	6.34%	4.89%	4.89%	4.89%	6.34%	5.74%	6.12%	6.49%	6.49%	6.50%	6.50%	6.87%	5.74%	6.12%	6.88%	7.30%	4.94%	6.51%	6.89%
Z01	5.60%	5.96%	7.50%	5.60%	5.97%	5.96%	5.96%	5.96%	5.96%	7.51%	5.38%	5.02%	6.11%	6.11%	5.37%	5.37%	5.73%	5.38%	5.02%	5.73%	6.12%	7.55%	6.90%	9.92%
Z02	4.57%	4.91%	6.35%	4.57%	4.92%	4.92%	4.91%	4.91%	4.91%	6.37%	4.34%	4.00%	5.02%	5.03%	4.34%	4.34%	4.67%	4.34%	4.00%	6.12%	5.04%	6.40%	5.76%	8.58%
Z03	8.34%	6.34%	6.34%	8.34%	8.78%	8.77%	6.34%	6.34%	7.11%	6.34%	5.02%	4.67%	5.74%	5.74%	5.02%	5.02%	5.37%	5.03%	4.67%	5.38%	5.03%	8.37%	5.03%	4.68%
Z04	8.34%	6.34%	6.34%	8.34%	8.78%	8.77%	6.34%	6.34%	7.11%	6.34%	5.02%	4.67%	5.74%	5.74%	5.02%	5.02%	5.37%	5.03%	4.67%	5.38%	5.03%	8.37%	5.03%	4.68%
Z07	8.07%	6.89%	7.67%	8.08%	8.50%	8.49%	6.89%	6.89%	6.89%	8.50%	11.21%	11.71%	12.18%	13.24%	11.70%	11.70%	12.68%	10.74%	11.22%	12.19%	11.22%	9.62%	12.23%	12.22%
Zi1	8.30%	7.08%	6.32%	8.30%	8.74%	8.73%	7.08%	7.08%	7.08%	8.74%	6.10%	6.48%	6.86%	7.67%	6.87%	6.87%	7.25%	6.87%	6.48%	7.26%	7.69%	10.66%	7.69%	6.49%
Zi2	7.41%	6.26%	5.54%	7.42%	7.83%	7.82%	6.26%	6.26%	6.26%	7.83%	4.96%	5.31%	5.66%	6.41%	5.67%	5.67%	6.79%	4.96%	5.31%	6.80%	6.42%	9.61%	6.42%	5.31%
Zi3	6.71%	5.60%	4.89%	6.71%	7.11%	7.10%	5.60%	5.60%	5.60%	7.11%	4.33%	4.67%	5.01%	5.73%	5.02%	5.02%	5.36%	5.02%	4.67%	5.37%	5.75%	8.83%	5.75%	4.67%
Zi4	7.10%	5.96%	7.49%	7.10%	7.51%	7.50%	5.96%	5.96%	5.96%	7.51%	4.67%	4.33%	5.37%	4.66%	4.67%	4.67%	5.01%	4.67%	4.33%	5.01%	5.38%	6.76%	5.38%	6.50%
Zi5	8.28%	7.06%	6.30%	8.28%	8.71%	8.71%	7.06%	7.06%	7.06%	8.71%	5.72%	6.09%	6.46%	7.25%	6.47%	6.47%	4.64%	6.47%	6.09%	6.85%	7.26%	10.64%	7.26%	6.09%
Zi6	5.24%	5.60%	7.10%	5.25%	5.60%	5.60%	5.60%	5.60%	5.60%	7.11%	5.02%	4.67%	5.74%	5.01%	5.02%	5.02%	5.37%	5.02%	4.67%	6.88%	5.75%	6.38%	5.75%	8.56%
Zi8	5.96%	4.89%	5.60%	5.96%	6.34%	6.34%	4.89%	4.89%	4.89%	6.34%	3.67%	3.99%	4.32%	5.01%	4.33%	4.33%	4.66%	4.33%	3.99%	4.67%	5.02%	7.98%	5.02%	5.38%
Zi0	5.60%	4.56%	5.96%	5.60%	5.97%	5.96%	4.56%	4.56%	4.56%	5.97%	3.99%	3.67%	4.66%	4.67%	3.99%	3.99%	4.32%	4.00%	3.67%	4.32%	4.67%	6.40%	4.67%	5.75%
CK1	34.21%	37.45%	35.25%	36.35%	35.34%	37.47%	35.25%	35.25%	37.44%	37.55%	40.02%	41.29%	42.35%	42.36%	42.47%	42.47%	43.56%	42.61%	41.29%	46.67%	42.63%	37.99%	37.72%	40.13%
CK2	25.27%	27.56%	26.00%	26.79%	26.06%	27.58%	26.01%	26.01%	27.55%	27.61%	29.29%	30.16%	30.96%	30.97%	31.01%	31.01%	26.75%	31.07%	30.16%	35.92%	27.66%	30.70%	32.99%	32.97%
CV1	31.87%	30.91%	32.88%	36.11%	35.09%	37.27%	32.88%	32.88%	32.88%	30.98%	26.59%	27.43%	28.18%	28.19%	28.24%	28.24%	29.01%	28.29%	27.43%	26.54%	30.10%	26.15	23.55%	22.14%
CL1	34.25%	40.02%	35.32%	34.30%	33.34%	33.28%	35.34%	35.34%	35.34%	33.34%	26.95%	27.79%	28.54%	28.56%	26.92%	26.92%	31.20%	26.97%	26.16%	32.26%	30.48%	31.76%	25.40%	26.98%

	G10	H01	J06	J07	K01	K03	K05	K06	L01	L02	L05	L06	L09	S01	T01	T02	T08	T12	U06	V03	W01	W03	W05
A01	22	18	22	20	14	13	13	16	22	12	15	16	16	11	20	10	14	13	15	11	24	25	12
A02	23	19	22	21	12	10	10	14	21	11	14	14	14	9	18	10	12	10	13	9	22	26	10
A04	26	19	26	24	12	14	14	16	22	14	17	17	17	12	20	14	15	14	16	12	25	27	13
B01	26	18	25	20	14	13	13	16	22	12	15	16	16	11	20	10	14	13	15	11	24	28	12
B02	26	17	26	21	15	14	14	15	22	13	14	17	17	12	20	10	15	14	16	12	25	28	13
B03	25	19	24	21	15	14	14	16	21	13	16	17	17	12	21	10	15	14	16	12	23	27	13
C01	23	16	22	21	12	10	10	14	21	11	14	14	14	9	18	10	12	10	13	9	22	26	10
C02	23	16	22	21	12	10	10	14	21	11	14	14	14	9	18	10	12	10	13	9	22	26	10
C04	23	16	22	21	12	10	10	14	21	11	14	14	14	9	18	10	12	10	13	9	22	26	10
C05	23	13	22	21	15	14	14	12	21	14	13	14	17	12	16	14	15	14	16	12	22	26	13
D01	10	13	9	2	16	15	15	10	9	10	6	11	16	14	11	12	16	15	16	14	16	15	14
D02	9	12	8	1	16	14	14	10	10	9	5	10	15	13	12	11	16	14	15	13	15	16	14
D03	11	14	10	3	18	16	16	12	10	11	8	13	17	15	13	14	18	16	17	15	17	16	16
D04	11	13	10	3	16	15	15	10	10	11	6	11	17	14	13	14	16	15	16	14	16	16	16
F02	8	13	8	0	16	15	15	10	10	10	6	11	16	14	13	12	16	15	16	14	16	16	14
F05	8	13	8	0	16	15	15	10	10	10	6	11	16	14	13	12	16	15	16	14	16	16	14
F06	10	14	10	4	17	16	16	11	11	10	7	12	16	14	14	13	17	16	18	14	16	17	15
F07	10	13	9	2	15	14	14	10	9	11	6	11	16	12	13	10	15	14	14	12	14	15	13
F10	9	14	8	1	16	14	14	11	10	10	7	12	15	13	14	11	16	14	15	13	15	16	14
G01	2	14	1	7	17	16	16	11	11	12	8	12	16	14	14	16	17	16	15	14	15	13	15
G02	11	14	10	3	18	16	16	9	12	11	8	10	17	15	14	14	18	16	17	15	14	18	16
G03	19	18	16	18	15	14	14	14	6	15	11	16	16	15	16	18	15	14	16	15	7	9	14
G04	14	5	14	9	15	14	14	4	10	12	0	7	17	12	5	12	15	14	16	12	16	16	14
G06	15	6	14	13	14	16	16	5	10	16	4	8	20	14	3	17	17	16	18	14	16	16	16
G10		15	2	8	19	17	17	13	13	14	10	14	18	16	15	17	19	17	16	16	16	11	16
H01	6.13%		14	13	17	16	16	5	12	14	4	8	18	14	6	17	17	16	18	14	16	17	16
J06	0.76%	5.76%		8	18	16	16	12	10	13	9	13	17	15	14	16	18	16	16	15	14	12	16
J07	3.03%	5.04%	2.73%		16	15	15	10	10	10	6	11	16	14	13	12	16	15	16	14	16	16	14
K01	8.09%	7.30%	7.68%	6.88%		2	2	15	14	5	9	16	7	3	17	6	3	2	4	3	16	16	2
K03	7.26%	6.51%	6.88%	6.11%	0.50%		0	14	12	4	8	14	5	2	16	4	2	0	2	2	14	15	1
K05	7.26%	6.51%	6.88%	6.11%	0.50%	0.00%		14	12	4	8	14	5	2	16	4	2	0	2	2	14	15	1
K06	5.02%	1.85%	4.67%	4.00%	6.11%	5.38%	5.38%		10	14	2	2	19	12	5	15	15	14	16	12	11	15	14
L01	5.07%	4.71%	4.04%	4.02%	5.45%	4.74%	4.74%	4.02%		15	7	11	15	12	10	14	14	12	14	12	4	3	13
L02	5.51%	5.90%	5.15%	3.75%	1.90%	1.33%	1.33%	5.89%	6.18%		10	15	1	4	16	4	5	4	6	4	17	18	3
L05	3.79%	1.31%	3.45%	2.20%	3.52%	2.88%	2.88%	0.77%	2.47%	3.79%		4	10	8	4	9	9	8	10	8	9	10	8
L06	5.37%	2.73%	5.02%	4.33%	6.49%	5.73%	5.73%	0.76%	4.36%	6.28%	1.62%		20	13	4	16	16	14	16	13	12	16	15
L09	7.69%	7.73%	7.29%	6.50%	2.43%	1.85%	1.85%	8.14%	6.18%	0.25%	4.16%	8.56%		6	19	8	6	5	7	7	20	20	4
S01	6.50%	5.78%	6.13%	5.40%	1.02%	0.50%	0.50%	4.69%	4.70%	1.32%	2.82%	5.03%	2.14%		14	3	3	2	4	0	16	16	2
T01	6.14%	2.16%	5.78%	5.06%	7.32%	6.52%	6.52%	1.86%	4.05%	6.70%	1.36%	1.58%	8.16%	5.77%		17	17	16	18	14	16	16	16
T02	7.28%	7.33%	6.90%	4.68%	2.14%	1.57%	1.57%	6.14%	5.42%	1.32%	3.46%	6.51%	2.73%	1.03%	7.32%		6	4	7	3	19	20	4
T08	8.10%	7.33%	7.70%	6.90%	1.02%	0.50%	0.50%	6.13%	5.44%	1.89%	3.53%	6.51%	2.14%	1.03%	7.32%	2.14%		2	4	3	16	16	2
T12	7.26%	6.51%	6.88%	6.11%	0.50%	0.00%	0.00%	5.38%	4.74%	1.33%	2.88%	5.73%	1.85%	0.50%	6.52%	1.57%	0.50%		2	2	14	15	1
U06	6.88%	7.74%	6.50%	6.51%	1.30%	0.76%	0.76%	6.52%	5.82%	2.19%	3.86%	6.90%	2.43%	1.30%	7.73%	2.43%	1.30%	0.76%		4	16	17	3
V03	6.49%	5.76%	6.11%	5.38%	1.02%	0.50%	0.50%	4.67%	4.70%	1.32%	2.82%	5.02%	2.43%	0.00%	5.78%	1.03%	1.02%	0.50%	1.30%		16	16	2
W01	6.79%	6.82%	5.67%	6.42%	6.40%	5.66%	5.66%	4.28%	1.30%	7.38%	3.45%	4.61%	8.43%	6.43%	6.84%	8.03%	6.42%	5.66%	6.81%	6.41%		7	15
W03	4.26%	7.19%	4.60%	6.79%	6.76%	6.01%	6.01%	6.04%	1.08%	7.85%	3.82%	6.40%	8.83%	6.79%	6.43%	8.41%	6.78%	6.01%	7.17%	6.77%	2.42%		16
W05	6.86%	6.90%	6.48%	5.74%	0.76%	0.25%	0.25%	5.74%	5.09%	1.06%	3.20%	6.11%	1.57%	0.76%	6.92%	1.29%	0.76%	0.25%	1.03%	0.76%	6.02%	6.38%	
Y02	7.67%	6.90%	7.27%	6.49%	0.76%	0.25%	0.25%	5.74%	4.73%	1.33%	2.88%	6.11%	1.57%	0.50%	6.51%	1.57%	0.51%	0.25%	0.76%	0.76%	6.02%	6.38%	0.50%
Y03	6.49%	9.04%	6.11%	5.39%	2.14%	1.57%	1.57%	7.72%	6.19%	1.33%	4.17%	8.13%	2.14%	2.14%	9.03%	1.57%	2.14%	1.57%	1.30%	2.14%	8.02%	8.41%	1.30%
Y06	8.09%	7.30%	7.69%	5.37%	1.02%	0.50%	0.50%	6.12%	4.74%	1.33%	2.89%	6.49%	2.43%	1.03%	7.33%	1.03%	1.03%	0.50%	1.30%	1.02%	6.40%	6.77%	0.76%
Y09	6.48%	6.51%	6.11%	5.37%	2.13%	1.57%	1.57%	5.38%	4.75%	2.48%	2.86%	4.32%	3.03%	2.13%	5.03%	3.34%	2.13%	1.57%	2.43%	2.13%	5.65%	6.01%	1.85%
Y10	7.68%	7.72%	7.28%	6.50%	1.29%	0.76%	0.76%	6.50%	4.74%	1.32%	3.52%	6.88%	1.57%	1.03%	6.51%	2.14%	1.03%	0.76%	1.30%	1.29%	6.79%	6.38%	1.02%
Z01	6.48%	9.94%	6.11%	5.37%	2.72%	2.13%	2.13%	8.56%	6.58%	1.04%	4.52%	8.99%	1.85%	2.44%	9.48%	1.85%	2.44%	2.13%	1.57%	2.73%	8.85%	9.26%	1.85%
Z02	6.88%	8.61%	6.50%	4.34%	1.85%	1.30%	1.30%	7.32%	5.82%	0.50%	3.84%	7.72%	1.30%	1.85%	8.59%	1.29%	1.85%	1.30%	2.14%	1.85%	7.62%	7.99%	1.03%
Z03	6.11%	6.13%	5.02%	5.02%	3.66%	4.32%	4.32%	5.75%	4.70%	3.76%	3.42%	6.12%	4.66%	3.67%	6.15%	4.33%	5.02%	4.32%	5.38%	3.66%	6.79%	7.17%	3.99%
Z04	6.11%	6.13%	5.02%	5.02%	3.66%	4.32%	4.32%	5.75%	4.70%	3.76%	3.42%	6.12%	4.66%	3.67%	6.15%	4.33%	5.02%	4.32%	5.38%	3.66%	6.79%	7.17%	3.99%
Z07	11.17%	13.85%	12.71%	11.70%	7.65%	6.88%	6.88%	11.21%	10.80%	5.91%	9.62%	11.69%	7.27%	6.15%	12.26%	6.89%	7.66%	6.88%	8.07%	6.15%	11.52%	11.97%	6.51%
Zi1	8.07%	8.98%	7.67%	6.87%	3.98%	4.65%	4.65%	7.69%	6.96%	4.43%	5.53%	8.09%	5.72%	3.98%	8.12%	4.65%	5.35%	4.65%	5.71%	3.98%	9.71%	9.23%	4.31%
Zi2	7.58%	7.62%	7.19%	5.67%	2.99%	3.62%	3.62%	6.41%	5.72%	4.03%	4.40%	6.80%	4.95%	3.00%	6.81%	3.62%	4.26%	3.62%	3.94%	3.00%	8.31%	7.88%	3.30%
Zi3	6.10%	6.90%	5.74%	5.02%	2.42%	3.03%	3.03%	5.74%	4.74%	2.51%	3.46%	6.11%	3.34%	2.15%	5.77%	2.75%	3.37%	3.03%	3.68%	2.43%	7.58%	7.16%	2.72%
Zi4	5.72%	6.51%	5.37%	4.67%	2.13%	1.57%	1.57%	5.38%	4.41%	1.61%	2.56%	5.73%	1.85%	1.86%	6.13%	3.04%	1.86%	1.57%	2.14%	2.13%	5.65%	6.01%	1.85%
Zi5	7.65%	8.52%	7.25%	6.47%	3.65%	4.30%	4.30%	7.25%	6.19%	3.77%	4.81%	7.65%	4.64%	3.35%	7.29%	4.00%	4.67%	4.30%	5.01%	3.65%	9.25%	8.79%	3.97%
Zi6	7.68%	8.58%	7.28%	5.02%	1.85%	1.29%	1.29%	7.30%	5.45%	0.77%	3.52%	7.70%	1.57%	1.57%	8.15%	1.57%	1.57%	1.29%	1.85%	1.85%	7.59%	7.97%	1.57%
Zi8	5.36%	6.13%	5.01%	4.33%	3.03%	2.43%	2.43%	5.02%	4.05%	1.91%	2.82%	5.37%	2.73%	1.58%	5.04%	2.15%	2.74%	2.43%	3.05%	1.85%	6.79%	6.39%	2.13%
Zi0	5.01%	5.75%	4.67%	3.99%	2.72%	2.13%	2.13%	4.67%	4.38%	1.62%	2.51%	5.02%	2.43%	1.30%	5.39%	1.86%	2.44%	2.13%	2.74%	1.57%	6.40%	6.77%	1.85%
CK1	46.36%	44.10%	45.21%	42.47%	30.24%	30.25%	30.25%	45.94%	33.41%	29.08%	31.81%	43.81%	30.34%	32.18%	37.72%	32.13%	32.08%	30.25%	33.02%	32.18%	40.38%	36.72%	31.14%
CK2	35.76%	36.11%	34.86%	31.01%	28.26%	28.27%	28.27%	31.55%	28.62%	26.46%	28.86%	33.85%	30.02%	26.74%	34.98%	26.72%	26.70%	28.27%	30.81%	26.75%	31.53%	32.30%	29.08%
CV1	26.45%	25.84%	25.74%	28.24%	28.04%	28.04%	28.04%	25.43%	23.71%	27.99%	22.32%	27.38%	29.90%	26.46%	23.54%	29.85%	26.39%	28.04%	30.71%	26.46%	29.57%	23.86%	28.90%
CL1	32.12%	27.85%	33.25%	26.92%	34.03%	34.04%	34.04%	30.93%	26.25%	30.97%	24.73%	33.29%	34.18%	32.16%	28.66%	32.11%	32.06%	34.04%	35.08%	32.16%	35.90%	32.58%	35.08%

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	Y02	Y03	Y06	Y09	Y10	Z01	Z02	Z03	Z04	Z07	Zi1	Zi2
A01	14	13	11	17	15	14	12	20	20	20	20	18
A02	11	14	12	15	13	15	13	16	16	17	17	16
A04	14	16	15	18	14	18	16	16	16	19	18	14
B01	14	13	11	17	15	14	12	20	20	20	20	18
B02	14	14	12	18	16	15	13	20	20	20	20	19
B03	14	14	12	18	16	15	13	20	20	20	20	19
C01	11	14	12	15	13	15	13	16	16	17	17	18
C02	11	14	12	15	13	15	13	16	16	17	17	18
C04	11	14	12	15	13	15	13	17	17	17	17	18
C05	14	16	15	18	16	18	16	18	18	20	20	19
D01	16	14	14	14	14	14	11	13	13	25	15	13
D02	15	13	13	13	15	13	10	12	12	26	16	14
D03	17	15	15	15	16	15	13	14	14	26	16	14
D04	16	15	14	14	16	15	13	14	14	28	18	16
F02	16	14	14	14	16	14	11	13	13	26	16	14
F05	16	14	14	14	16	14	11	13	13	26	16	14
F06	16	14	14	14	16	14	12	14	14	27	17	16
F07	14	14	12	12	14	14	11	13	13	24	18	13
F10	15	13	13	13	15	13	10	12	12	25	16	14
G01	16	14	17	14	16	14	15	14	14	26	17	18
G02	17	15	15	15	17	15	13	13	13	25	18	16
G03	14	18	15	16	13	18	16	20	20	22	23	22
G04	14	15	12	14	16	16	14	13	13	26	18	16
G06	16	20	17	16	16	22	20	12	12	26	16	14
G10	18	16	19	16	16	16	16	15	15	25	19	18
H01	16	20	17	16	18	22	20	15	15	28	20	18
J06	17	15	18	15	17	15	16	13	13	27	18	17
J07	16	14	14	14	16	14	11	13	13	26	16	14
K01	2	6	3	6	4	8	5	10	10	19	10	8
K03	1	4	2	4	2	6	4	11	11	17	12	10
K05	1	4	2	4	2	6	4	11	11	17	12	10
K06	14	18	15	14	16	20	17	14	14	25	18	16
L01	12	15	12	12	12	16	14	12	12	24	16	14
L02	4	4	4	7	4	3	2	10	10	15	11	10
L05	8	10	8	8	9	11	10	9	9	22	14	11
L06	15	19	16	11	16	20	18	15	15	26	19	16
L09	4	6	7	8	4	5	4	12	12	18	14	13
S01	2	6	3	6	3	7	5	10	10	16	10	8
T01	16	20	17	13	16	21	20	15	15	26	19	16
T02	4	4	3	9	6	5	4	11	11	17	12	10
T08	2	6	3	6	3	7	5	13	13	19	14	11
T12	1	4	2	4	2	6	4	11	11	17	12	10
U06	2	4	4	7	4	4	6	14	14	20	14	10
V03	2	6	3	6	4	8	5	10	10	16	10	8
W01	15	19	16	14	16	20	18	16	16	26	22	20
W03	16	20	16	15	16	21	19	17	17	26	21	19
W05	2	4	2	5	3	5	3	10	10	16	11	9
Y02		4	2	5	2	5	4	12	12	18	13	10
Y03	1.57%		3	9	6	1	2	13	13	20	14	13
Y06	0.76%	1.03%		6	4	4	2	13	13	19	14	11
Y09	1.85%	3.35%	2.13%		5	9	7	11	11	20	12	10
Y10	0.50%	2.14%	1.29%	1.85%		5	4	14	14	16	13	10
Z01	1.85%	0.25%	1.57%	3.34%	1.85%		2	14	14	20	15	14
Z02	1.30%	0.76%	0.76%	2.43%	1.30%	0.51%		12	12	18	13	12
Z03	4.66%	5.02%	5.01%	4.32%	5.37%	5.73%	4.67%		0	22	8	9
Z04	4.66%	5.02%	5.01%	4.32%	5.37%	5.73%	4.67%	0.00%		22	8	9
Z07	7.26%	8.48%	7.66%	8.47%	6.51%	8.47%	7.28%	9.78%	9.78%		23	21
Zi1	4.99%	5.35%	5.35%	4.65%	4.99%	6.08%	5.00%	3.03%	3.03%	10.19%		8
Zi2	3.62%	4.94%	4.26%	3.62%	3.62%	5.30%	4.60%	3.30%	3.30%	8.80%	2.99%	
Zi3	2.72%	3.36%	3.66%	3.03%	2.72%	3.66%	3.05%	2.13%	2.13%	8.06%	1.85%	1.28%
Zi4	1.29%	2.44%	2.13%	1.02%	1.29%	2.13%	1.58%	3.66%	3.66%	8.47%	3.98%	3.30%
Zi5	3.97%	4.66%	4.99%	4.30%	3.97%	4.99%	4.33%	3.33%	3.33%	9.28%	3.03%	2.39%
Zi6	1.02%	1.02%	0.76%	2.43%	1.02%	0.76%	0.25%	5.37%	5.37%	8.06%	5.71%	4.95%
Zi8	2.13%	2.74%	3.03%	2.43%	2.13%	3.03%	2.44%	2.73%	2.73%	7.27%	2.42%	1.83%
Zi0	1.85%	2.44%	2.72%	2.13%	2.43%	2.72%	2.15%	2.43%	2.43%	7.66%	2.72%	2.11%
CK1	30.24%	30.23%	28.54%	29.41%	30.29%	31.18%	29.41%	32.24%	32.24%	30.74%	31.08%	30.63%
CK2	28.26%	28.25%	26.70%	30.84%	28.29%	29.11%	27.49%	28.35%	28.35%	26.22%	25.88%	28.54%
CV1	28.04%	31.66%	29.81%	30.78%	28.08%	32.72%	30.77%	24.93%	24.93%	32.93%	25.56%	25.13%
CL1	34.03%	34.01%	32.06%	37.36%	34.09%	35.14%	33.08%	32.19%	32.19%	38.74%	30.98%	32.48%

	Zi3	Zi4	Zi5	Zi6	Zi8	Zi0	CK1	CK2	CV1	CL1
A01	16	17	20	14	15	14	55	47	52	54
A02	14	15	17	14	13	12	57	50	51	58
A04	13	18	16	17	14	15	56	48	52	55
B01	16	17	20	14	15	14	56	49	55	54
B02	17	18	20	14	16	15	56	48	54	53
B03	17	18	20	14	16	15	57	50	56	53
C01	14	15	17	14	13	12	56	48	52	55
C02	14	15	17	14	13	12	56	48	52	55
C04	14	15	17	14	13	12	57	50	52	55
C05	17	18	20	17	16	15	57	50	51	53
D01	11	12	14	13	10	10	58	51	47	48
D02	12	11	15	12	10	10	59	52	48	49
D03	13	14	16	14	11	12	60	52	49	50
D04	14	12	17	13	13	12	60	52	49	50
F02	13	12	16	13	11	10	60	52	49	48
F05	13	12	16	13	11	10	60	52	49	48
F06	14	13	12	14	12	11	61	49	50	52
F07	13	12	16	13	11	10	60	52	49	48
F10	12	11	15	12	10	10	59	52	48	47
G01	14	13	16	16	12	11	62	56	47	52
G02	14	14	17	14	13	12	60	50	50	51
G03	20	16	23	16	19	20	58	52	47	52
G04	14	14	17	14	13	12	57	54	44	46
G06	12	16	15	20	14	14	58	54	43	48
G10	15	14	18	18	14	13	62	56	47	52
H01	16	16	20	20	15	14	61	56	46	49
J06	14	14	17	17	13	12	62	56	46	53
J07	13	12	16	13	11	10	60	52	49	48
K01	7	6	10	5	8	8	52	50	49	54
K03	8	4	11	4	7	6	52	50	49	54
K05	8	4	11	4	7	6	52	50	49	54
K06	14	14	17	17	13	12	62	53	46	52
L01	12	11	15	14	10	11	54	50	44	47
L02	7	4	10	2	5	4	50	48	48	51
L05	9	7	12	9	8	7	52	50	43	46
L06	15	14	18	18	14	13	61	55	48	53
L09	9	5	12	4	8	7	52	52	50	54
S01	6	5	9	4	4	4	53	49	47	52
T01	14	15	17	19	13	14	57	56	44	50
T02	8	8	10	4	6	5	53	49	50	52
T08	9	5	12	4	8	7	53	49	47	52
T12	8	4	11	4	7	6	52	50	49	54
U06	10	6	13	5	8	8	54	52	51	55
V03	7	6	10	5	5	4	53	49	47	52
W01	18	14	21	18	16	16	59	53	50	56
W03	17	15	20	19	16	16	57	54	45	53
W05	8	5	10	4	6	5	52	51	50	55
Y02	8	4	10	3	6	5	52	50	49	54
Y03	9	7	12	3	8	7	52	50	52	54
Y06	10	6	13	2	8	8	50	49	50	52
Y09	8	3	11	7	7	6	51	52	51	56
Y10	8	4	10	3	6	7	52	50	49	54
Z01	10	6	13	2	8	8	52	51	52	55
Z02	8	4	11	1	7	6	51	50	51	53
Z03	6	10	9	14	8	7	53	50	46	52
Z04	6	10	9	14	8	7	53	50	46	52
Z07	20	20	22	20	18	19	52	48	53	58
Zi1	5	10	8	14	7	8	52	48	46	52
Zi2	4	9	7	13	5	6	52	50	46	52
Zi3		5	3	9	2	2	52	48	46	52
Zi4	1.85%		8	4	4	3	52	50	49	54
Zi5	1.02%	3.02%		12	4	5	55	47	50	56
Zi6	3.34%	1.29%	4.64%		8	7	50	49	50	52
Zi8	0.50%	1.29%	1.56%	2.72%		1	52	48	46	52
Zi0	0.76%	1.02%	1.84%	2.43%	0.25%		53	49	47	52
CK1	31.25%	30.29%	33.50%	28.57%	31.24%	32.18%		22	47	49
CK2	25.97%	28.28%	24.92%	26.72%	25.97%	26.73%	9.77%		48	47
CV1	25.67%	28.09%	29.36%	29.85%	25.67%	26.46%	29.81%	31.05%		22
CL1	31.16%	34.08%	35.53%	32.10%	31.16%	32.13%	30.98%	29.24%	9.67%	

Table A4 Pairwise (HKY85 Γ-corrected) sequence divergences of a 420 bp long fragment of CR-I among haplotypes found in South African Cape hares and some Eurasian outgroup species. Coding of haplotypes as in chapter 6 Table 6.1; eur = *Lepus europaeus*; swi = *L. capensis swinhoei* ; man = *L. mandshuricus*; oio = *L. oiostolus*.

	HT1	HT2	HT3	HT4	HT5	HT6	HT7	HT8	HT9	HT10	HT11	HT12	HT13	HT14	HT15	HT16	HT17	HT18
HT1		30	45	47	47	50	46	49	52	48	51	45	48	48	49	47	51	46
HT2	9.95%		50	48	50	49	50	50	49	48	52	51	49	49	50	50	47	49
HT3	16.61%	20.15%		21	20	12	35	23	25	28	22	22	14	22	23	23	32	22
HT4	17.70%	18.61%	6.36%		3	17	34	4	25	22	21	13	17	1	2	6	33	5
HT5	17.88%	20.21%	6.06%	0.75%		14	31	7	25	23	20	12	14	4	5	3	32	2
HT6	19.83%	19.45%	3.31%	4.91%	3.95%		31	17	21	24	18	20	6	18	19	17	28	16
HT7	17.07%	19.96%	12.40%	11.77%	10.44%	10.42%		34	38	37	32	31	29	35	36	32	18	31
HT8	19.00%	19.98%	7.14%	1.01%	1.81%	4.91%	11.77%		29	24	25	15	19	3	4	10	33	9
HT9	21.21%	19.42%	8.03%	7.94%	8.04%	6.42%	14.00%	9.70%		29	15	31	23	26	27	26	31	25
HT10	18.43%	18.72%	9.10%	6.58%	7.03%	7.42%	13.10%	7.35%	9.53%		24	23	26	23	24	24	26	25
HT11	20.81%	21.95%	6.73%	6.28%	5.98%	5.25%	10.78%	7.85%	4.23%	7.34%		26	20	22	23	23	29	22
HT12	16.61%	20.85%	6.81%	3.59%	3.31%	6.05%	10.42%	4.23%	10.74%	7.03%	8.34%		20	14	15	13	32	12
HT13	18.49%	19.45%	3.95%	4.91%	3.96%	1.55%	9.51%	5.62%	7.21%	8.24%	5.97%	6.05%		18	19	17	28	16
HT14	18.34%	19.28%	6.75%	0.25%	1.00%	5.26%	12.28%	0.75%	8.37%	6.96%	6.66%	3.90%	5.26%		1	7	34	6
HT15	18.96%	19.93%	7.13%	0.50%	1.27%	5.62%	12.78%	1.01%	8.79%	7.34%	7.04%	4.23%	5.62%	0.25%		8	35	7
HT16	17.81%	20.10%	7.21%	1.54%	0.75%	4.96%	10.88%	2.67%	8.44%	7.41%	7.12%	3.62%	4.96%	1.81%	2.09%		33	1
HT17	20.23%	17.87%	10.81%	11.19%	10.83%	8.99%	5.23%	11.19%	10.33%	7.96%	9.32%	10.80%	8.99%	11.68%	12.16%	11.28%		32
HT18	17.22%	19.45%	6.82%	1.27%	0.50%	4.62%	10.42%	2.38%	8.03%	7.83%	6.73%	3.31%	4.62%	1.54%	1.81%	0.25%	10.81%	
HT19	19.03%	16.77%	10.44%	10.81%	9.53%	7.81%	3.61%	10.81%	11.90%	9.29%	8.98%	9.51%	7.81%	11.29%	11.77%	9.96%	2.37%	9.52%
HT20	19.20%	17.50%	7.64%	4.92%	5.33%	5.33%	11.40%	6.37%	8.04%	6.65%	6.74%	6.82%	5.33%	5.27%	5.62%	5.68%	9.89%	5.33%
HT21	19.88%	19.50%	3.96%	4.92%	3.96%	1.55%	10.44%	5.62%	6.43%	8.25%	5.25%	6.82%	1.55%	5.27%	5.62%	4.97%	9.89%	4.62%
HT22	16.58%	17.43%	3.00%	4.57%	4.28%	3.00%	10.88%	5.98%	6.04%	7.82%	4.90%	4.96%	2.40%	4.91%	5.25%	4.61%	7.73%	4.28%
HT23	16.55%	17.40%	3.00%	4.56%	4.28%	3.00%	9.93%	5.98%	6.03%	7.81%	4.90%	4.95%	2.40%	4.90%	5.25%	4.61%	8.54%	4.28%
HT24	22.57%	18.60%	4.57%	5.56%	4.57%	1.54%	11.27%	6.29%	5.61%	8.15%	4.51%	7.53%	2.67%	5.92%	6.29%	5.61%	9.78%	5.26%
HT25	20.45%	20.06%	7.20%	6.73%	6.42%	4.96%	8.61%	8.36%	4.61%	9.52%	3.58%	8.88%	4.96%	7.12%	7.52%	7.60%	9.85%	7.20%
HT26	19.19%	21.69%	6.06%	6.75%	6.83%	5.32%	13.49%	7.55%	7.22%	9.11%	6.35%	7.62%	6.83%	7.15%	7.54%	8.04%	11.82%	7.63%
HT27	21.21%	23.92%	6.81%	6.36%	6.05%	4.62%	11.37%	7.13%	10.74%	7.41%	7.52%	6.81%	4.62%	6.74%	7.13%	6.42%	11.77%	6.81%
HT28	20.54%	23.17%	7.63%	5.62%	5.33%	5.32%	11.39%	7.14%	10.75%	7.83%	7.53%	6.81%	4.62%	5.99%	6.36%	6.43%	12.82%	6.05%
HT29	13.41%	18.31%	5.26%	3.55%	3.28%	5.25%	8.53%	4.19%	10.61%	7.73%	8.25%	2.67%	5.26%	3.87%	3.55%	3.58%	9.76%	3.27%
HT30	15.35%	16.71%	14.64%	15.63%	15.20%	14.63%	18.00%	15.62%	15.70%	15.69%	15.32%	16.32%	15.74%	15.08%	15.05%	15.16%	17.27%	14.64%
HT31	22.01%	21.62%	5.68%	4.57%	4.29%	3.00%	10.90%	5.99%	7.61%	7.03%	5.61%	5.67%	3.00%	4.91%	5.26%	5.32%	10.34%	4.97%
HT32	25.51%	23.47%	8.77%	10.01%	9.66%	7.92%	13.23%	10.95%	7.49%	11.24%	5.53%	10.88%	8.77%	9.55%	10.00%	10.10%	11.59%	9.65%
HT33	21.89%	18.69%	8.44%	7.93%	7.61%	5.31%	10.87%	9.68%	5.67%	8.22%	4.56%	9.32%	6.04%	8.36%	8.78%	8.01%	8.54%	7.60%
eur	38.71%	39.28%	38.52%	38.30%	38.59%	38.51%	32.09%	38.29%	44.50%	36.21%	41.28%	42.03%	38.52%	39.42%	38.19%	40.72%	33.90%	39.64%
swi	45.08%	47.24%	42.82%	41.45%	38.35%	38.24%	41.19%	39.19%	45.35%	44.41%	50.36%	36.07%	38.24%	40.30%	41.36%	39.24%	38.91%	38.23%
man	35.41%	45.72%	37.67%	34.35%	31.76%	35.56%	33.28%	36.41%	37.76%	31.21%	39.38%	31.58%	35.54%	35.34%	36.31%	32.54%	29.73%	31.68%
oio	42.67%	47.22%	41.84%	36.15%	33.49%	35.34%	38.03%	38.19%	34.29%	35.67%	38.79%	34.31%	37.37%	37.17%	36.09%	32.50%	34.91%	31.67%

	HT19	HT20	HT21	HT22	HT23	HT24	HT25	HT26	HT27	HT28	HT29	HT30	HT31	HT32	HT33	eur	swi	man	oio
HT1	49	49	50	45	45	54	51	49	52	51	39	43	53	58	53	78	86	76	84
HT2	45	46	49	46	46	48	50	52	55	54	47	45	52	55	48	78	87	84	87
HT3	31	24	14	11	11	16	23	20	22	24	18	42	19	27	26	79	84	78	83
HT4	32	17	17	16	16	19	22	22	21	19	13	44	16	30	25	79	83	75	78
HT5	29	18	14	15	15	16	21	22	20	18	12	43	15	29	24	79	80	72	75
HT6	25	18	6	11	11	6	17	18	16	18	18	42	11	25	18	79	80	76	77
HT7	13	33	31	32	30	33	27	37	33	33	27	48	32	37	32	73	83	74	80
HT8	32	21	19	20	20	21	26	24	23	23	15	44	20	32	29	79	81	77	80
HT9	34	25	21	20	20	19	16	23	31	31	31	44	24	24	19	84	86	78	76
HT10	29	22	26	25	25	26	29	28	24	25	25	44	23	33	26	77	86	72	78
HT11	28	22	18	17	17	16	13	21	24	24	26	43	19	19	16	81	89	79	80
HT12	29	22	22	17	17	24	27	24	22	22	10	45	19	32	28	82	78	72	76
HT13	25	18	6	9	9	10	17	22	16	16	18	44	11	27	20	79	80	76	79
HT14	33	18	18	17	17	20	23	23	22	20	14	43	17	29	26	80	82	76	79
HT15	34	19	19	18	18	21	24	24	23	21	13	43	18	30	27	79	83	77	78
HT16	30	19	17	16	16	19	24	25	21	21	13	43	18	30	25	81	81	73	74
HT17	9	30	30	25	27	30	30	34	34	36	30	47	31	34	27	75	81	70	77
HT18	29	18	16	15	15	18	23	24	22	20	12	42	17	29	24	80	80	72	73
HT19		29	27	26	24	27	29	33	31	33	27	47	28	33	28	77	81	70	78
HT20	9.53%		18	17	17	20	23	24	22	18	18	42	17	31	24	81	89	81	80
HT21	8.66%	5.33%		11	11	4	15	18	16	16	22	44	11	23	16	83	82	76	79
HT22	8.21%	4.97%	3.00%		2	13	20	17	21	21	15	40	14	20	19	79	80	72	77
HT23	7.39%	4.96%	3.00%	0.50%		13	20	17	21	21	15	41	14	22	19	78	81	71	78
HT24	8.56%	5.99%	1.00%	3.59%	3.58%		15	20	20	20	24	44	13	23	16	81	82	74	77
HT25	9.50%	7.21%	4.28%	6.03%	6.03%	4.23%		25	23	23	27	41	18	22	17	86	86	86	79
HT26	11.43%	7.64%	5.33%	4.97%	4.96%	5.99%	8.03%		16	20	24	46	13	25	22	84	84	76	79
HT27	10.42%	6.82%	4.62%	6.42%	6.41%	5.98%	7.19%	4.62%		10	22	48	7	25	22	83	86	78	79
HT28	11.40%	5.33%	4.62%	6.43%	6.42%	5.98%	7.20%	6.06%	2.70%		20	46	7	27	20	84	89	79	82
HT29	8.54%	5.26%	6.74%	4.23%	4.23%	7.45%	8.77%	7.53%	6.73%	5.98%		41	19	31	28	72	81	72	74
HT30	17.40%	14.66%	15.76%	13.57%	14.06%	15.61%	14.06%	16.93%	18.12%	16.92%	13.99%		46	48	41	76	82	81	76
HT31	9.08%	4.97%	3.00%	3.95%	3.95%	3.59%	5.31%	3.63%	1.83%	1.83%	5.61%	16.92%		20	15	84	84	76	79
HT32	11.21%	10.60%	7.12%	5.97%	6.71%	7.03%	6.71%	7.92%	7.91%	8.77%	10.46%	17.85%	5.97%		17	86	85	82	80
HT33	9.06%	7.61%	4.62%	5.67%	5.66%	4.56%	4.95%	6.81%	6.80%	6.04%	9.20%	14.06%	4.28%	4.89%		84	82	76	75
eur	36.01%	40.92%	43.42%	38.46%	37.27%	41.40%	47.06%	44.71%	43.13%	44.54%	31.75%	36.48%	44.55%	46.39%	44.38%		55	49	54
swi	39.09%	49.91%	40.58%	38.13%	39.14%	40.99%	45.24%	43.00%	45.29%	49.65%	39.65%	39.77%	42.85%	42.72%	40.29%	21.88%		36	28
man	29.85%	41.30%	35.66%	31.65%	30.71%	33.99%	39.89%	35.71%	37.60%	38.79%	31.90%	40.45%	35.53%	41.08%	35.46%	18.83%	12.40%		34
oio	36.04%	38.53%	37.42%	35.27%	36.19%	35.69%	37.23%	37.40%	37.31%	40.68%	32.76%	33.98%	37.36%	37.37%	33.30%	20.90%	8.72%	11.18%	

Table A5 Pairwise (HKY85Γ) sequence divergences of a 420 bp long fragment of CR-I among scrub and Cape hares and a few Eurasian outgroup species. Coding of individuals as in Table A1; eur = *Lepus europaeus*; man = *L. mandshuricus*.

	A02	B01	C01	D01	D02	F02	G01	H01	I01	J03	J06	K01	K05	L01	L06	M01	N01	O01	P01
A02		8	4	26	25	28	24	24	68	64	25	16	15	25	20	67	60	66	56
B01	2.85%		4	22	21	24	24	20	69	63	25	14	13	22	18	66	59	65	55
C01	1.36%	1.37%		24	23	26	22	20	67	61	23	14	13	23	18	64	57	63	53
D01	11.09%	8.94%	9.99%		1	4	14	18	72	66	15	18	17	14	14	71	62	66	59
D02	10.53%	8.43%	9.46%	0.33%		3	13	17	73	67	14	17	16	15	13	72	63	67	60
F02	12.24%	9.99%	11.08%	1.37%	1.02%		14	20	72	66	15	20	19	18	16	71	62	66	59
G01	10.00%	10.00%	8.95%	5.30%	4.87%	5.31%		14	72	68	1	20	19	15	12	73	66	66	63
H01	10.01%	7.96%	7.95%	7.15%	6.67%	8.13%	5.32%		71	67	15	18	17	14	8	72	65	67	64
I01	42.08%	43.46%	40.78%	46.90%	48.42%	47.40%	47.59%	45.67%		12	71	71	71	65	72	22	22	14	23
J03	37.12%	36.00%	33.81%	38.94%	40.17%	39.30%	41.96%	40.29%	4.43%		67	65	65	61	68	18	20	6	17
J06	10.54%	10.54%	9.47%	5.75%	5.30%	5.75%	0.33%	5.76%	46.10%	40.66%		21	20	14	13	72	67	65	64
K01	6.06%	5.18%	5.18%	7.13%	6.66%	8.11%	8.11%	7.14%	45.31%	37.65%	8.62%		1	14	14	68	63	67	58
K05	5.62%	4.76%	4.76%	6.66%	6.19%	7.62%	7.62%	6.67%	45.32%	37.66%	8.11%	0.33%		13	13	68	63	67	58
L01	10.55%	8.97%	9.47%	5.32%	5.76%	7.17%	5.77%	5.31%	37.89%	33.50%	5.33%	5.31%	4.88%		11	66	63	59	60
L06	7.93%	6.98%	6.98%	5.30%	4.87%	6.20%	4.45%	2.84%	46.84%	41.36%	4.87%	5.30%	4.87%	4.04%		71	66	68	63
M01	41.14%	39.87%	37.43%	45.96%	47.44%	46.36%	49.71%	47.64%	9.01%	7.02%	48.14%	41.62%	41.62%	39.35%	45.88%		26	20	23
N01	32.80%	31.81%	29.88%	34.46%	35.54%	34.79%	39.46%	37.87%	9.13%	8.09%	40.69%	35.41%	35.42%	35.59%	38.89%	11.19%		20	13
O01	39.53%	38.33%	36.00%	38.95%	40.18%	39.32%	39.43%	40.31%	5.29%	2.07%	38.22%	40.04%	40.05%	31.53%	41.37%	7.99%	8.10%		17
P01	28.88%	28.01%	26.30%	31.33%	32.31%	31.60%	35.82%	36.61%	9.66%	6.64%	36.94%	30.31%	30.32%	32.33%	35.36%	9.54%	4.84%	6.64%	
P05	29.85%	28.94%	27.18%	32.36%	33.38%	32.64%	37.02%	35.56%	9.14%	6.18%	38.19%	31.31%	31.31%	32.39%	36.53%	9.02%	4.42%	6.18%	0.33%
Q08	34.80%	33.75%	31.71%	38.82%	40.05%	39.21%	39.32%	45.52%	9.13%	6.18%	38.12%	39.91%	39.92%	34.47%	43.85%	10.07%	9.12%	6.18%	8.60%
R01	33.07%	32.07%	30.09%	28.81%	29.73%	29.07%	34.03%	31.68%	7.03%	7.03%	35.11%	33.58%	33.59%	29.80%	32.57%	11.07%	3.56%	7.03%	4.79%
S01	5.21%	4.37%	4.37%	6.22%	5.76%	7.16%	7.16%	6.23%	46.86%	38.89%	7.64%	0.67%	0.33%	4.87%	4.45%	43.07%	36.57%	41.38%	31.29%
T01	10.05%	10.06%	9.00%	6.70%	7.18%	8.68%	5.78%	3.26%	44.13%	38.97%	6.24%	7.66%	7.17%	4.89%	2.47%	43.27%	34.42%	38.97%	35.43%
U06	6.53%	5.64%	5.64%	6.67%	6.21%	7.64%	7.63%	7.65%	45.30%	37.64%	8.13%	1.01%	0.67%	5.75%	5.75%	44.29%	35.40%	40.03%	30.30%
V03	5.62%	4.76%	4.76%	5.74%	5.30%	6.67%	6.67%	5.75%	46.87%	38.90%	7.14%	1.01%	0.67%	4.45%	4.03%	43.07%	36.58%	41.39%	31.29%
W01	10.94%	9.86%	9.86%	9.02%	8.51%	10.08%	8.01%	8.02%	43.47%	38.46%	7.52%	6.12%	5.67%	2.10%	5.24%	42.49%	40.89%	36.21%	35.01%
X01	37.06%	37.06%	34.84%	36.97%	38.12%	38.01%	38.15%	39.42%	21.51%	21.50%	36.99%	31.62%	31.63%	29.94%	36.93%	22.50%	23.91%	23.09%	22.26%
Y02	6.53%	5.63%	5.63%	6.67%	6.21%	7.62%	8.63%	7.64%	43.24%	35.87%	9.15%	1.01%	0.67%	5.30%	5.75%	39.66%	33.75%	38.18%	28.81%
Z05	38.29%	37.13%	34.87%	42.69%	44.05%	43.13%	46.09%	44.22%	7.61%	4.01%	44.65%	41.26%	41.27%	36.68%	45.37%	7.50%	7.59%	4.01%	7.11%
Z07	10.13%	10.13%	9.11%	13.48%	14.09%	15.35%	14.11%	16.07%	43.99%	36.52%	14.74%	9.08%	8.59%	12.35%	13.48%	36.87%	34.33%	38.85%	30.32%
Zim1	8.42%	7.45%	7.45%	5.30%	5.74%	7.14%	8.12%	9.16%	43.93%	36.52%	8.63%	3.62%	4.03%	6.68%	7.13%	40.43%	34.33%	38.83%	29.39%
CK1	47.52%	43.25%	43.24%	49.76%	51.36%	50.25%	53.84%	48.44%	10.77%	8.60%	52.15%	42.46%	42.46%	40.14%	49.71%	5.65%	14.43%	9.66%	11.33%
CV1	33.50%	35.69%	33.50%	34.13%	35.21%	36.65%	32.42%	32.11%	27.19%	26.24%	34.13%	35.21%	36.65%	32.42%	32.11%	29.67%	28.15%	26.23%	27.13%
CL1	38.73%	34.18%	34.17%	32.70%	33.73%	30.97%	33.04%	32.75%	22.35%	26.74%	32.70%	33.73%	30.97%	33.04%	32.75%	28.09%	22.34%	24.91%	23.98%
eur	39.81%	43.48%	39.81%	41.52%	42.75%	41.87%	42.11%	40.53%	52.18%	52.18%	43.36%	42.79%	44.05%	41.89%	39.20%	56.05%	57.05%	52.18%	55.23%
man	42.43%	41.20%	40.04%	49.52%	48.04%	47.01%	47.28%	45.39%	51.06%	48.15%	48.76%	46.52%	47.91%	48.23%	49.53%	53.48%	42.66%	48.12%	43.99%

	P05	Q08	R01	S01	T01	U06	V03	W01	X01	Y02	Z05	Z07	Zim1	CK1	CV1	CL1	eur	man
A02	57	62	60	14	24	17	15	26	64	17	65	25	21	72	60	65	68	71
B01	56	61	59	12	24	15	13	24	64	15	64	25	19	69	62	61	71	70
C01	54	59	57	12	22	15	13	24	62	15	62	23	19	69	60	61	68	69
D01	60	66	56	16	17	17	15	22	64	17	69	31	14	74	61	60	70	76
D02	61	67	57	15	18	16	14	21	65	16	70	32	15	75	62	61	71	75
F02	60	66	56	18	21	19	17	24	65	19	69	34	18	74	63	58	70	74
G01	64	66	61	18	15	19	17	20	65	21	71	32	20	76	59	60	70	74
H01	63	71	59	16	9	19	15	20	66	19	70	35	22	73	59	60	69	73
I01	22	22	18	72	70	71	72	70	45	69	19	70	70	25	50	45	79	78
J03	16	16	18	66	66	65	66	66	45	63	11	64	64	21	49	50	79	76
J06	65	65	62	19	16	20	18	19	64	22	70	33	21	75	58	61	71	75
K01	59	67	61	2	19	3	3	16	59	3	68	23	10	69	61	62	71	74
K05	59	67	61	1	18	2	2	15	59	2	68	22	11	69	61	62	72	75
L01	60	62	57	13	13	15	12	6	57	14	64	29	17	67	56	57	70	75
L06	64	70	60	12	7	15	11	14	64	15	71	31	18	74	61	64	68	76
M01	22	24	26	69	69	70	69	69	46	66	19	64	67	15	52	51	81	79
N01	12	22	10	64	62	63	64	68	48	61	19	62	62	31	51	45	82	72
O01	16	16	18	68	66	67	68	64	47	65	11	66	66	23	49	48	79	76
P01	1	21	13	59	63	58	59	63	46	56	18	58	57	26	50	47	81	73
P05		22	12	60	62	59	60	64	45	57	17	57	58	25	49	46	80	72
Q08	9.13%		22	68	70	67	68	62	51	65	7	64	64	31	55	52	83	78
R01	4.37%	9.01%		62	58	61	62	62	44	59	19	60	60	31	47	39	72	72
S01	32.32%	41.24%	34.69%		17	3	1	16	60	2	69	21	10	70	60	61	71	74
T01	34.41%	44.00%	30.64%	6.69%		20	16	21	62	19	69	31	21	72	56	60	69	78
U06	31.30%	39.90%	33.57%	1.01%	8.15%		4	17	61	3	68	24	13	71	63	62	74	77
V03	32.33%	41.25%	34.70%	0.33%	6.24%	1.36%		17	60	4	69	22	9	70	60	61	71	73
W01	36.16%	33.99%	34.32%	6.14%	8.56%	6.60%	6.58%		60	17	65	32	24	74	63	66	75	81
X01	21.51%	26.48%	20.58%	32.77%	34.96%	33.74%	32.66%	32.36%		59	49	62	58	47	48	46	75	81
Y02	29.77%	38.07%	31.97%	0.67%	7.65%	1.01%	1.36%	6.58%	31.56%		66	24	11	67	59	62	73	76
Z05	6.65%	2.44%	7.51%	42.65%	42.75%	41.25%	42.65%	37.30%	24.77%	39.36%		65	67	24	52	53	84	76
Z07	29.45%	36.44%	32.54%	8.12%	13.55%	9.60%	8.60%	13.93%	33.86%	9.60%	37.65%		27	68	66	66	70	74
Zi1	30.36%	36.41%	32.55%	3.63%	8.66%	4.87%	3.23%	10.07%	30.67%	4.03%	40.02%	11.18%		68	58	61	68	72
CK1	10.77%	14.42%	14.25%	43.88%	46.89%	45.14%	43.89%	48.99%	23.03%	40.47%	10.21%	41.23%	41.22%		49	52	84	78
CV1	26.24%	32.36%	24.68%	33.03%	29.20%	36.22%	33.04%	35.87%	24.51%	32.34%	29.20%	39.10%	31.01%	26.20%		30	78	84
CL1	23.17%	28.62%	18.01%	33.68%	32.70%	34.69%	33.69%	38.85%	22.54%	35.13%	29.71%	38.55%	33.64%	28.62%	13.35%		78	76
eur	53.74%	58.58%	42.87%	42.97%	40.65%	46.84%	42.84%	47.61%	47.94%	45.97%	60.55%	41.03%	39.28%	60.48%	53.34%	52.50%		49
man	42.81%	50.96%	43.12%	46.72%	53.13%	51.02%	45.24%	56.75%	54.89%	50.00%	48.11%	45.16%	44.01%	51.19%	62.33%	47.94%	25.36%	

Table A6 Alignment of 1020 bp of cytochrome *b* retrieved from African and European hares. L.sax = *Lepus saxatilis*; L. cap = *L. capensis*; L. corsic = *L. corsicanus*; L. granat = *L. granatensis*; L. mandshu = *L. mandshuricus*; SW = southwestern; C = central; N = northern; sc = south-central; locality coding as in Table A1.

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L.sax SW-A     CTAATAATTCAAATCCTAACTGGCCTATTTCTAGCCATACACTATACATCAGATACAGCA
L.sax SW-B     CTAATAATTCAAATCCTAACTGGCCTATTTCTAGCCATACACTATACATCAGACACAGCA
L.sax C-D      CTAATGATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.sax C-E      CTAATGATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.sax N-T      CTAATAATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.sax N-Y      CTAATAATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.sax N-Zi     CTAATAATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.sax N-Z      CTAATAATTCAAATCCTAACTGGCCTATTCCTAGCCATACACTACACATCAGACACAGCA
L.cap N-Q      CTAATAATCCAAATCCTAACTGGCCTATTCTTAGCTATACACTACACATCAGACACAGCA
L.cap N-J1     CTAATAATCCAAATCCTAACTGGCCTGTTCCTAGCTATACACTACACATCAGACACAGCA
L.cap N-J2     CTAATAATCCAAATCCTAACTGGCCTGTTCCTAGCTATACACTACACATCAGACACAGCA
L.cap N-O      CTAATAATCCAAATCCTAACTGGCCTATTCCTAGCTATACACTACACATCAGACACAGCA
L.cap N-N      CTAATAATCCAAATCCTAACTGGCCTGTTCCTAGCTATACACTACACATCAGACACAGCA
L.cap SC-CL    CTAATAATCCAAATCCTAACTGGCCTATTTCTAGCTATACACTATACATCAGACACAGCA
L.cap SC-CV    CTAATAATCCAAATCCTAACTGGCCTATTTCTAGCTATACACTATACATCAGACACAGCA
L.CAP ACHK1    CTAATAATCCAAATCCTAACTGGACTATTCCTAGCTATACACTACACATCAGACACAGCA
L.corsic       CTAATAATCCAAATCCTAACTGGCCTGTTCCTAGCTATACACTACACATCAGATACAGCA
L.timidus      CTAATAATCCAAATCCTAACTGGCCTATTCCTAGCTATACACTACACATCAGACACAGCA
L.granat       CTTATAATCCAAATCCTAACTGGCTTGTTCCTAGCTATACACTATACATCAGACACAGCA
L.mandshu      CTAATGATTCAAATCCTGACTGGACTATTCCTAGCTATACACTATACATCAGATACAACA

L.sax SW-A     ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAACTACGGCTGACTCATCCGT
L.sax SW-B     ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAACTACGGCTGACTCATCCGT
L.sax C-D      ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.sax C-E      ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.sax N-T      ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.sax N-Y      ACAGCATTCTCCTCAGTTACACATATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.sax N-Zi     ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.sax N-Z      ACAGCATTCTCCTCAGTTACACACATTTGTCGAGACGTAAATTACGGCTGACTCATCCGT
L.cap N-Q      ACAGCGTTCTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap N-J1     ACAGCGTTTTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap N-J2     ACAGCGTTCTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap N-O      ACAGCGTTTTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap N-N      ACAGCGTTCTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap SC-CL    ACAGCATTCTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.cap SC-CV    ACAGCATTCTCCTCAGTCACACATATTTGTCGAGACGTAAACTACGGTTGACTTATCCGT
L.CAP ACHK1    ACAGCGTTCTCCTCAGTCACACATATTTGTCGAGACGTAAATTACGGTTGACTTATCCGT
L.corsic       ACAGCATTTTCTTCAGTCACACATATTTGCCGAGACGTAAACCATGGCTGACTTATTCGT
L.timidus      ACAGCATTTTCTTCAGTCACACATATTTGTCGAGACGTAAACTATGGCTGACTTATTCGT
L.granat       ACAGCATTCTCCTCAGTCACTCATATTTGCCGAGACGTAAACTACGGCTGACTCATTCGC
L.mandshu      ACAGCATTCTCCTCAGTCACACATATTTGCCGAGATGTAAACTACGGCCGACTTATCCGC

L.sax SW-A     TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCCTATATATACATGTGGGCCGT
L.sax SW-B     TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTGGGCCGT
L.sax C-D      TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.sax C-E      TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.sax N-T      TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.sax N-Y      TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.sax N-Zi     TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.sax N-Z      TATCTACACGCTAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap N-Q      TACCTACACGCCAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap N-J1     TACCTACACGCCAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap N-J2     TACCTACACGCCAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap N-O      TACCTACACGCCAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap N-N      TACCTACACGCCAACGGAGCATCAATATTCTTTATTTGCTTATATATACATGTAGGCCGT
L.cap SC-CL    TACCTACACGCTAACGGAGCATCGATATTCTTTGTCTGCTTATATATACATGTAGGCCGT
L.cap SC-CV    TACCTTCACGCTAACGGAGCATCAATATTCTTCGTCTGCTTATATATACATGTAGGCCGT
L.CAP ACHK1    TACCTACACGCTAACGGAGCATCAATATTCTTTGTTTGCTTATACATACATGTAGGCCGT
L.corsic       TACCTACACGCCAACGGAGCATCAATATTTTTTATCTGCTTATATATACATGTAGGTCGT
L.timidus      TACCTGCACGCCAACGGAGCATCAATATTTTTTATCTGCTTATATATACATGTAGGTCGT
L.granat       TACCTGCACGCTAACGGAGCTTCAATATTCTTTATTTGCTTATACATACATGTAGGCCGT
L.mandshu      TATCTGCACGCTAACGGAGCATCAATATTCTTTATCTGCCTATACATACATGTAGGCCAT
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L.sax SW-A     GGAATCTATTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATTTTATTA
L.sax SW-B     GGAATCTATTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATTTTATTA
L.sax C-D      GGAATCTACTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.sax C-E      GGAATCTACTACGGCTCATATACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.sax N-T      GGAATCTACTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.sax N-Y      GGAATCTACTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.sax N-Zi     GGAATCTACTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.sax N-Z      GGAATCTACTACGGCTCATACACTTATCTCGAAACCTGAAATATTGGCATTATCCTATTA
L.cap N-Q      GGAATCTACTACGGCTCATACACTTATCTAGAAACCTGAAACATTGGCATTATTCTGTTA
L.cap N-J1     GGAATCTACTACGGCTCATACACTTATCTAGAAACCTGAAACATTGGCATTATTCTGTTA
L.cap N-J2     GGAATCTACTACGGCTCATACACTTATCTAGAAACCTGAAACATTGGCATTATTCTGTTA
L.cap N-O      GGAATCTACTACGGCTCATACACTTACCTAGAAACCTGAAACATTGGCATTGTTCTGTTA
L.cap N-N      GGAATCTACTACGGCTCATACACTTATCTAGAAACCTGAAACATTGGCATTATTCTGTTA
L.cap SC-CL    GGAATCTACTACGGCTCATATACTTACCTAGAAACCTGAAACATTGGCATTATTCTGCTA
L.cap SC-CV    GGAATCTACTACGGCTCATATACTTACCTAGAAACCTGAAACATTGGCATTATTCTGCTA
L.CAP ACHK1    GGAATCTACTACGGCTCATATACTTATCTTGAAACTTGAAACATTGGCATTATTCTGTTA
L.corsic       GGAATCTACTATGGCTCATATACTTACCTAGAAACCTGGAATATTGGCATTATTCTTCTA
L.timidus      GGAATCTACTACGGCTCATATACTTACCTAGAAACCTGGAATATTGGCATTATTCTACTA
L.granat       GGAATCTACTACGGCTCATATACCTATCTAGAAACCTGAAATATTGGCATTATCCTATTA
L.mandshu      GGAATCTACTACGGCTCATACACTTACCTAGAAACCTGGAATATTGGCATTATCCTGCTA

L.sax SW-A     TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGACAAATATCTTTC
L.sax SW-B     TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGACAAATATCTTTC
L.sax C-D      TTTGCAGTAATAGCCACGGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.sax C-E      TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.sax N-T      TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.sax N-Y      TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.sax N-Zi     TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.sax N-Z      TTTGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTCCCATGAGGGCAAATATCTTTC
L.cap N-Q      TTCGCAGTAATAGCAACAGCGTTCATAGGCTATGTCCTCCCATGAGGGCAAATATCATTC
L.cap N-J1     TTCGCAGTAATAGCAACAGCGTTCATAGGCTATGTCCTCCCATGAGGGCAAATATCATTC
L.cap N-J2     TTCGCAGTAATAGCAACAGCGTTCATAGGCTATGTCCTCCCATGAGGGCAAATATCATTC
L.cap N-O      TTCGCAGTAATAGCAACAGCGTTCATAGGCTATGTCCTCCCATGAGGGCAAATATCATTC
L.cap N-N      TTCGCAGTAANAGCAACAGCGTTCATAGGCTATGTCCTCCCATGGGGGCAAATATCATTC
L.cap SC-CL    TTCGCAGTAATAGCAACAGCATTCATAGGCTACGTCCTCCCATGAGGACAAATATCATTC
L.cap SC-CV    TTCGCGGTAATAGCAACAGCATTCATAGGCTACGTCCTCCCATGAGGACAAATATCATTC
L.CAP ACHK1    TTCGCAGTAATAGCAACAGCATTCATAGGCTACGTCCTCCCATGAGGGCAAATATCATTC
L.corsic       TTTGCAGTAATAGCCACAGCATTTATAGGCTATGTCCTCCCATGAGGACAAATATCATTC
L.timidus      TTTGCAGTAATAGCCACAGCATTTATAGGCTATGTTCTCCCATGAGGACAAATATCATTC
L.granat       TTTGCAGTAATAGCCACAGCATTCATAGGCTACGTCCTTCCATGAGGACAAATATCATTC
L.mandshu      TTCGCAGTAATAGCCACAGCATTCATAGGCTATGTCCTTCCATGAGGACAAATATCATTC

L.sax SW-A     TGAGGAGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax SW-B     TGAGGAGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax C-D      TGGGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax C-E      TGGGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax N-T      TGAGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax N-Y      TGAGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax N-Zi     TGAGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACCCTA
L.sax N-Z      TGGGGGGCCACCGTAATTACTAATCTTCTATCAGCCATCCCCTACATTGGAACAACTCTA
L.cap N-Q      TGAGGTGCCACCGTAATTACTAATCTTCTATCAGCCATTCCTTACATCGGAACAACCCTA
L.cap N-J1     TGAGGTGCCACCGTAATTACTAATCTTCTATCAGCCATTCCTTACATCGGAACAACCCTA
L.cap N-J2     TGAGGTGCCACCGTAATTACTAATCTTCTATCAGCCATTCCTTACATCGGAACAACCCTA
L.cap N-O      TGAGGTGCCACCGTAATTACTAATCTTCTATCAGCCATTCCTTACATCGGAACAACCCTA
L.cap N-N      TGAGGTGCCACCGTAATTACTAATCTTCTATCAGCCATTCCTTACATCGGAACAACCCTA
L.cap SC-CL    TGAGGTGCCACCGTGATTACTAATCTTCTATCAGCCATCCCTTACATTGGAACAACCCTA
L.cap SC-CV    TGAGGTGCCACCGTGATTACTAATCTTCTATCAGCCATCCCTTACATTGGAACAACCCTA
L.CAP ACHK1    TGAGGCGCCACCGTAATCACTAATCTTCTATCAGCCATTCCTTACATTGGAACAACCTTA
L.corsic       TGAGGCGCTACTGTAATTACTAATCTTTTATCAGCTATCCCCTACATTGGAACAACCCTA
L.timidus      TGAGGCGCTACTGTAATTACCAATCTTTTATCAGCTATCCCCTACATTGGAACAACCCTA
L.granat       TGAGGAGCTACCGTGATTACTAATCTCCTATCAGCCATCCCCTATATCGGAACAACCTTA
L.mandshu      TGAGGAGCTACCGTAATTACTAACTTCCTATCAGCCATCCCCTACATCGGAACAACTCTA
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L.sax SW-A     GTTGAATGGATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTTACCCGATTCTTCGCT
L.sax SW-B     GTTGAATGGATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTTACCCGATTCTTCGCT
L.sax C-D      GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTCACCCGATTCTTCGCT
L.sax C-E      GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTCACCCGATTCTTCGCT
L.sax N-T      GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTCACCCGATTTTTCGCT
L.sax N-Y      GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTCACCCGATTTTTCGCT
L.sax N-Zi     GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTTACCCGATTTTTCGCT
L.sax N-Z      GTTGAATGAATCTGAGGGGGATTTTCAGTTGACAAAGCCACACTTACCCGATTTTTCGCT
L.cap N-Q      GTTGAATGAATCTGAGGGGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap N-J1     GTTGAATGAATCTGAGGAGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap N-J2     GTTGAATGAATCTGAGGAGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap N-O      GTTGAATGAATCTGAGGAGGATTTTCAGTTGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap N-N      GTTGAATGAATCTGAGGAGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap SC-CL    GTTGAATGAATCTGAGGAGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.cap SC-CV    GTTGAATGAATCTGAGGAGGATTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.CAP ACHK1    GTTGAATGAATCTGAGGAGGTTTTTCAGTCGACAAAGCCACACTAACCCGATTCTTCGCT
L.corsic       GTTGAATGAATTTGAGGAGGGTTCTCAGTCGACAAAGCTACACTCACCCGATTCTTCGCT
L.timidus      GTTGAATGAATTTGAGGAGGATTTTCAGTCGACAAAGCTACACTCACCCGATTCTTCGCT
L.granat       GTTGAATGAATCTGAGGAGGGTTTTCAGTTGATAAAGCCACACTTACCCGATTCTTCGCT
L.mandshu      GTTGAATGAATCTCAGGAGGATTTTCAGTTGATAAAGCCACACTTACCCGATTCTTCGCA

L.sax SW-A     TTCCACTTCATTCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTTCTC
L.sax SW-B     TTCCACTTCATTCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTTCTC
L.sax C-D      TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.sax C-E      TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.sax N-T      TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.sax N-Y      TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.sax N-Zi     TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.sax N-Z      TTCCACTTCATCCTCCCATTTATCATCGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.cap N-Q      TTCCATTTCATCCTACCATTTATCATCGCAGCTCTAGTAATAATCCATTTACTTTTCCTC
L.cap N-J1     TTCCATTTCATCCTACCATTTATCATCGCAGCTCTAGTAATAATCCATTTACTTTTCCTC
L.cap N-J2     TTCCATTTCATCCTACCATTCATCATCGCAGCTCTAGTAATAATCCATTTACTTTTCCTC
L.cap N-O      TTCCATTTCATCCTACCATTTATCATCGCAGCTCTAGTAATAATCCATTTACTTTTCCTC
L.cap N-N      TTCCATTTCATCCTACCATTTATCATCGCAGCTCTAGTAATAATCCATTTACTTTTCCTC
L.cap SC-CL    TTCCACTTTATCCTACCATTTATCATTGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.cap SC-CV    TTCCACTTCATCCTACCATTTATCATTGCAGCACTAGTAATAATCCATTTACTTTTCCTC
L.CAP ACHK1    TTCCACTTTATCTTACCATTCATCATTGCAGCACTAGTAATAATCCATCTACTTTTCCTC
L.corsic       TTCCACTTCATTCTCCCATTCATCATCGCAGCACTAGTGATGATTCACCTACTTTTCCTC
L.timidus      TTCCACTTCATTCTCCCATTCATCATCGCAGCATTAGTGATGATTCACTTACTTTTCCTC
L.granat       TTCCACTTCATTCTTCCATTCATTATTGCAGCACTAGTAATAATTCACTTACTTTTTCTT
L.mandshu      TTCCACTTCATCCTACCATTCATCATTGCAGCACTAGTCATAGTCCACTTACTTTTCCTC

L.sax SW-A     CATGAAACTGGCTCCAATAACCCATCAGGCATTCCGTCAGACTCCGATAAAATTCCATTC
L.sax SW-B     CATGAAACTGGCTCCAATAACCCATCAGGCATTCCGTCAGACTCCGATAAAATTCCATTC
L.sax C-D      CATGAAACTGGCTCCAATAACCCATCAGGCATTCCGTCAGACTCCGACAAAATTCCATTC
L.sax C-E      CATGAAACTGGCTCCAATAACCCATCAGGCATTCCGTCAGACTCCGACAAAATTCCATTC
L.sax N-T      CATGAAACTGGCTCCAATAACCCATCAGGCATTCCATCAGACTCCGACAAAATTCCATTC
L.sax N-Y      CATGAAACTGGCTCCAATAACCCATCAGGCATTCCATCAGACTCCGACAAAATTCCATTC
L.sax N-Zi     CATGAAACTGGCTCCAATAACCCATCAGGCATTCCATCAGACTCCGACAAAATTCCATTC
L.sax N-Z      CATGAAACTGGCTCCAATAACCCATCAGGCATTCCGTCAGACTCCGACAAAATTCCATTC
L.cap N-Q      CATGAAACCGGCTCTAATAATCCATCGGGAATCCCATCAGACTCTGATAAAATCCCATTT
L.cap N-J1     CATGAAACCGGTTCTAATAATCCATCAGGAATCCCATCAGACTCTGATAAAATCCCATTT
L.cap N-J2     CATGAAACCGGTTCTAACAATCCATCGGGAATTCCATCAGACTCTGATAAAATCCCATTC
L.cap N-O      CATGAAACCGGTTCTAATAATCCATCGGGAATCCCATCAGACTCTGATAAAATCCCATTT
L.cap N-N      CATGAAACCGGTTCTAATAACCCATCAGGAATTCCATCAGACTCTGATAAAATCCCATTT
L.cap SC-CL    CATGAAACCGGTTCTAATAATCCATCGGGAATCCCATCAGACTCCGATAAAATCCCATTC
L.cap SC-CV    CATGAAACCGGTTCTAATAATCCATCAGGAATCCCATCAGACTCCGATAAAATCCCATTT
L.CAP ACHK1    CATGAAACTGGTTCTAATAACCCATCGGGAATCCCATCAGACTCCGATAAAATCCCATTT
L.corsic       CATGAAACTGGCTCCAATAATCCATCAGGTATCCCATCAGACTCTGATAAGATTCCATTC
L.timidus      CATGAAACTGGCTCCAACAACCCATCAGGTATCCCATCAGACTCTGATAAGATTCCATTC
L.granat       CATGAAACTGGCTCCAATAACCCATCAGGCATCCCATCAGACTCTGATAAAATTCCATTC
L.mandshu      CACAAAACTGGCTCTAACAACCCATCAGGCATCCCATGGGACTCCGATAAAATTCCCTTC
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L.sax SW-A     CACCCCTACTATACAATCAAAGATGTTCTAGGATTTCTAATACTCATTCTCCTACTTATA
L.sax SW-B     CACCCCTACTATACAATCAAAGATGTTCTAGGATTTCTAATACTCATTCTCCTACTTATA
L.sax C-D      CACCCTTATTATACAATCAAAGATGTTCTAGGGTTTCTAGTACTTATTCTCCTACTTATA
L.sax C-E      CACCCTTATTATACAATCAAAGATGTTCTAGGGTTTCTAGTACTTATTCTCCTACTTATA
L.sax N-T      CACCCTTATTATACAATCAAAGATATTCTAGGGTTTCTAGTTCTTATTCTCCTACTTATA
L.sax N-Y      CACCCTTATTATACAATCAAAGATATTCTAGGGTTTCTAGTTCTTATTCTCCTACTTATA
L.sax N-Zi     CACCCTTATTATACAATCAAAGATGTTCTAGGGTTTCTAGTTCTTATTCTCCTACTTATA
L.sax N-Z      CACCCTTATTATACAATCAAAGATGTTCTAGGGTTTCTAGTACTTATTCTCCTACTTATA
L.cap N-Q      CACCCCTATTATACAATTAAAGATGTCCTAGGATTTCTTATCCTTATCCTCCTACTCACA
L.cap N-J1     CACCCCTATTATACAATTAAAGATGTCCTAGGATTTCTTATCCTTATCCTCCTACTCACA
L.cap N-J2     CACCCCTATTATACAATTAAAGATGTCCTAGGATTTCTTATCCTTATCCTCCTACTCACA
L.cap N-O      CACCCCTATTATACAATTAAAGATGTCCTAGGATTTCTTATCGTTATCTTCCTACTCACA
L.cap N-N      CACCCCTACTATACAATTAAAGATCTCCTAGGATTTCTTATCCTTATCCTCCTACTCACA
L.cap SC-CL    CACCCCTATTATACAATTAAAGACGTCCTAGGATTTCTTATCCTTATCCTCCTACTGATA
L.cap SC-CV    CACCCCTATTATACAATTAAAGACGTCCTAGGATTTCTTATCCTCATCCTCCTACTGATA
L.CAP ACHK1    CACCCCTATTATACAATTAAAGACGTCCTAGGATTTCTCATCCTTATCCTCCTACTTATA
L.corsic       CACCCCTATTACACAATTAAAGACCTTCTAGGATTTCTCGTACTTATCCTCCTACTCATA
L.timidus      CACCCCTATTACACAATTAAAGACCTTCTAGGATTTCTCGTACTTATCCTCCTACTTATA
L.granat       CATCCCTACTACACAATTAAAGATGCTCTTGGGTTTCTTATACTTATTCTCCTACTCATA
L.mandshu      CATCCCTACTACACAATCAAGGACACCCTAGGATTTCTTATCCTAATCCTCCTACTTCTA

L.sax SW-A     CTCCTAGTCTTATTCTNCCCTGACCTTCTTGGAGACCCAGACAACTACACCCCTGCCAAC
L.sax SW-B     CTCCTAGTCTTATTCTCCCCTGACCTTCTTGGAGACCCAGACAACTACACCCCTGCCAAC
L.sax C-D      CTCCTAGTCCTATTCTCCCCTGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.sax C-E      CTCCTAGTCCTATTCTCCCCTGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.sax N-T      CTCCTAGTCCTATTCTCCCCTGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.sax N-Y      CTCCTAGTCCTATTCTCCCCTGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.sax N-Zi     CTCCTAGTCCTATTCTCCCCTGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.sax N-Z      CTCCTAGTCCTATTCTCCCCCGACCTTCTTGGCGACCCAGACAACTACACCCCTGCCAAC
L.cap N-Q      CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAC
L.cap N-J1     CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAC
L.cap N-J2     CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAC
L.cap N-O      CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAC
L.cap N-N      CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAC
L.cap SC-CL    CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGTGACCCAGACAACTACACCCCTGCCAAC
L.cap SC-CV    CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAACTACACCCCTGCCAAC
L.CAP ACHK1    CTTCTAGTCTTATTCTCCCCTGACCTTCTCGGCGACCCAGACAATTACACCCCTGCCAAT
L.corsic       CTCCTAGTCTTATTCTCCCCTGATCTTCTCGGAGACCCAGACAATTACACCCCTGCCAAT
L.timidus      CTCCTAGTCTTATTCTCCCCTGACCTTCTCGGAGACCCAGACAACTACACCCCTGCCAAT
L.granat       CTCCTAGTCTTATTCTCTCCTGACCTTCTCGGCGACCCAGACAACTATACCCCTGCCAAT
L.mandshu      CTCCTAGTTCTATTCTCCCCTGATCTACTCGGAGACCCAGACAACTATACCCCTGCCAAT

L.sax SW-A     CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGGTATTTCCTATTTGCCTATGCCATC
L.sax SW-B     CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGGTATTTCCTATTTGCCTATGCCATC
L.sax C-D      CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.sax C-E      CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.sax N-T      CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.sax N-Y      CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.sax N-Zi     CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.sax N-Z      CCCCTCAACACTCCCCCTCACATCAAACCTGAGTGATATTTCCTATTTGCCTATGCCATT
L.cap N-Q      CCCCTCAACACTCCTCCTCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.cap N-J1     CCCCTCAACACTCCTCCTCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.cap N-J2     CCCCTCAACACTCCTCCTCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.cap N-O      CCCCTCAACACTCCTCCCCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.cap N-N      CCCCTCAACACTCCTCCTCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.cap SC-CL    CCTCTCAACACTCCTCCTCATATTAAACCTGAATGATATTTCCTATTTGCTTATGCTATC
L.cap SC-CV    CCTCTCAACACTCCTCCTCATATTAAACCTGAATGATATTTCCTATTTGCCTATGCCATC
L.CAP ACHK1    CCTCTCAACACTCCTCCTCACATTAAACCTGAATGATACTTCCTATTTGCCTATGCCATC
L.corsic       CCTCTCAACACCCCTCCCCATATCAAACCTGAATGGTATTTTCTATTTGCCTACGCCATT
L.timidus      CCTCTCAACACTCCTCCCCACATCAAACCTGAATGGTATTTTCTATTTGCCTACGCCATT
L.granat       CCCCTCAACACCCCTCCCCACATCAAACCTGAATGATACTTCCTATTTGCCTATGCCATT
L.mandshu      CCCCTCAATACTCCTCCTCACATCAAACCCGAATGATACTTCCTATTTGCCTACGCCATT
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L.sax SW-A     TTACGCTCCATTCCCAACAAGCTAGGAGGTGTTATAGCCCTAGTTATATCAATTCTCATC
L.sax SW-B     TTACGCTCCATTCCCAACAAGCTAGGAGGTGTTATAGCCCTAGTTATATCAATTCTCATC
L.sax C-D      TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.sax C-E      TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.sax N-T      TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.sax N-Y      TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.sax N-Zi     TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.sax N-Z      TTACGCTCCATTCCCAACAAGCTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.cap N-Q      CTACGCTCTATTCCTAATAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATCCTCATC
L.cap N-J1     CTACGCTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATCCTCATC
L.cap N-J2     CTACGCTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATCCTCATC
L.cap N-O      CTACGCTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATCCTCATC
L.cap N-N      CTACGCTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATCCTCATC
L.cap SC-CL    CTACGTTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTTATC
L.cap SC-CV    CTACGTTCTATCCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTTATC
L.CAP ACHK1    CTACGCTCTATTCCTAACAAACTTGGAGGTGTTCTAGCCCTAGTTATATCAATTCTTATC
L.corsic       TTACGCTCCATCCCTAACAAACTAGGAGGCGTTCTAGCCCTAGTTATGTCAATTCTCATC
L.timidus      TTACGCTCCATCCCTAACAAACTAGGAGGTGTTCTAGCCCTAGTTATATCAATTCTCATC
L.granat       TTGCGCTCCATCCCTAATAAACTAGGAGGCGTCCTAGCCCTGGTTATATCAATTCTTATC
L.mandshu      TTACGTTCTATCCCTAACAAACTAGGAGGTGTTCTAGCTCTAGTCATATCAATCCTTATC

L.sax SW-A     CTAGCAGTCATCCCCTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCCATC
L.sax SW-B     CTAGCAGTCATCCCCTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCCATC
L.sax C-D      CTAGCAGTCATCCCTTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCTATC
L.sax C-E      CTAGCAGTCATCCCTTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCTATC
L.sax N-T      CTAGCAGTCATCCCTTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCTATC
L.sax N-Y      CTAGCAGTCATCCCTTTCCTCCATATATCCAAACAACGCAGCATAATATTCCGACCTATC
L.sax N-Zi     CTAGCAGTCATCCCTTTCCTCCACATATCCAAACAACGCAGCATAATATTTCGACCTATC
L.sax N-Z      CTAGCAGTCATCCCTTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCTATC
L.cap N-Q      CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGTAGCATAATATTTCGACCCATC
L.cap N-J1     CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGTAGCATAATATTTCGACCCATC
L.cap N-J2     CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGTAGCATAATATTTCGACCCATC
L.cap N-O      CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGTAGCATAACATTTCGACCCATC
L.cap N-N      CTAGCAATTATCCCTCTCCTCCACATATCCAAACAACGTAGCATAATATTTCGACCCATC
L.cap SC-CL    CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGCAGCATGATATTTCGACCCATC
L.cap SC-CV    CTAGCAATCATCCCTCTCCTCCACATATCCAAACAACGCAGCATGATATTTCGACCCATC
L.CAP ACHK1    CTAGCAATCTTCCCTCTCCTCCACATATCCAAACAACGTAGCATAATATTTCGACCCATC
L.corsic       CTAGCAATTATCCCCTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCCATT
L.timidus      CTAGCAATTATCCCCTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCGATT
L.granat       CTAGCAATTTTCCCCTTCCTCCACATATCCAAACAACGTAGCATAATATTCCGACCTATT
L.mandshu      CTAGCAATTATCCCCTTCCTCCACATATCCAAACAACGCAGCATAATATTCCGACCCATT

L.sax SW-A     AGTCAAGTCCTTTTCTGGGCCCTTGTCGCAGATCTTCTCACACTCACATGAATCGGAGGA
L.sax SW-B     AGTCAAGTCCTTTTCTGGGCCCTTGTCGCAGATCTTCTCACACTCACATGAATCGGAGGA
L.sax C-D      AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.sax C-E      AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.sax N-T      AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.sax N-Y      AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.sax N-Zi     AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.sax N-Z      AGCCAAGTCCTTTTCTGAGTCCTCGTCGCAGATCTTCTCACACTCACATGAATTGGAGGA
L.cap N-Q      AGTCAAGTTCTTTTCTGAATTCTCGTCGCAGACCTTCTTACACTTACATGAATTGGAGGG
L.cap N-J1     AGTCAAGTTCTTTTCTGAATTCTCGTCGCAGACCTTCTTACACTTACATGAATTGGAGGA
L.cap N-J2     AGTCAAGTTCTTTTCTGAATTCTCGTCGCAGACCTTCTTACACTTACATGAATTGGAGGA
L.cap N-O      AGTCAAGTTCTTTTCTGAGTTCTCGTCGCAGACCTTCTTACACTTACATGAATTGGAGGA
L.cap N-N      AGTCAAGTCCTTTTCTGAGTTCTCGTCGCAGACCTTCTTACACTTACATGAATTGGAGGA
L.cap SC-CL    AGTCAAGTTCTTTTCTGAGTTCTCGTCGCAGACCTTCTTACACTCACATGAATTGGGGGA
L.cap SC-CV    AGTCAAGTTCTTTTCTGAGTTCTCGTCGCAGACCTTCTTACACTCACATGAATTGGAGGA
L.CAP ACHK1    AGTCAAGTTCTTTTCTGAATTCTCGTCGCAGACCTTCTTACACTCACATGAATTGGAGGA
L.corsic       AGCCAAGTCCTCTTCTGAATTCTCGTTGCAGACCTTCTGACACTCACATGGATTGGAGGA
L.timidus      AGCCAAGTTCTCTTCTGAATCCTCATTGCAGACCTTCTAACACTCACATGGATCGGAGGA
L.granat       AGCCAAGTTCTTTTCTGAATCCTCGTTGCAGACCTTCTTACACTTACATGGATCGGAGGA
L.mandshu      AGCCAAGTCCTTTTTTGAATCCTTGTCGCAGACCTTCTGACACTCACATGAATTGGAGGG
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L.sax SW-A     CAACCAGTCGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax SW-B     CAACCAGTCGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax C-D      CAACCAGTTGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax C-E      CAACCAGTTGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax N-T      CAACCAGTTGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax N-Y      CAACCAGTTGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax N-Zi     CAACCAGTTGAACACCCATTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.sax N-Z      CAACCAGTTGAACACCCGTTTATCACTATCGGACAAGTAGCATCCATTCTCTACTTCTCC
L.cap N-Q      CAACCAGTTGAGCACCCATTTATTACCATTGGACAAGTAGCATCTATCCTCTATTTCTCC
L.cap N-J1     CAACCAGTTGAACACCCATTCATTACCATTGGACAAGTAGCATCTATCCTCTATTTCTCC
L.cap N-J2     CAACCAGTTGAACACCCATTTATTACCATTGGACAAGTAGCATCTATCCTCTATTTCTCC
L.cap N-O      CAACCAGTTGAACACCCATTCATTACCATTGGACAAGTAGCATCTATCCTCTATTTCTCC
L.cap N-N      CAACCAGTTGAACACCCATTTATTACCATTGGACAAGTAGCATCTATCCTCTATTTCTCC
L.cap SC-CL    CAACCAGTTGAACACCCATTTATCACCATCGGACAAATAGCATCTATCCTCTATTTCTCC
L.cap SC-CV    CAACCAGTTGAACACCCATTTATTACCATTGGACAAATAGCATCTATCCTCTATTTCTCC
L.CAP ACHK1    CAGCCAGTTGAACACCCATTTATTACCATTGGACAGATAGCGTCTATCCTCTATTTCTCC
L.corsic       CAACCAGTTGAACACCCATTTATTACTATTGGACAAGTAGCATCTATCCTTTACTTCTCT
L.timidus      CAACCAGTTGAACACCCATTTATTACTATTGGACAAGTAGCATCTATCCTCTACTTCTCT
L.granat       CAACCAGTTGAACACCCCTTTATCACTATTGGACAAGTAGCATCAATTCTTTACTTCACC
L.mandshu      CAACCAGTTGAACACCCATTTATTACTATTGGTCAAGTGGCATCTATCCTTTACTTCTCC

L.sax SW-A     ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax SW-B     ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax C-D      ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax C-E      ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax N-T      ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax N-Y      ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax N-Zi     ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.sax N-Z      ATTATCCTTATCCTCATGCCCCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap N-Q      ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap N-J1     ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap N-J2     ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap N-O      ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap N-N      ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap SC-CL    ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAGAATAAAATCCTCAAATGAAGG
L.cap SC-CV    ATCATTCTTATCTTCATACCTCTCGCAAGCTTAATCGAAAATAAAATCCTCAAATGAAGG
L.CAP ACHK1    ATCATTCTTATCTTCATACCTCTCGCAAGCTTGATCGAGAATAAAATCCTCAAATGAAGG
L.corsic       ATTATCCTCATCCTTATACCTCTTGCAAGCTTAATTGAGAATAAAATCCTTAAATGAAGG
L.timidus      ATCATCCTTATCCTTATACCCCTTGCAAGCTTAATTGAAAATAAAATCCTTAAATGAAGG
L.granat       ATCATCCTCATCCTTATACCCCTCGCAAGCTTAGTCGAGAATAAAATCCTCAAATGAAGG
L.mandshu      ATCATTCTCATCCTCATGCCCCTCGCAAGCTTAGTCGAAAATAAAATCCTTAAATGAAGG
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Table A7 Protein Translations of the cytochrome *b* sequences in Table A6. Coding of individuals as in Table A6.

Individual	Sequence
L.sax SW-A	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax SW-B	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax C-D	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax C-E	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax N-T	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax N-Y	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax N-Zi	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.sax N-Z	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap N-Q	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap N-J1	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap N-J2	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap N-O	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap N-N	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F I C L Y M H V G R
L.cap SC-CL	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F V C L Y M H V G R
L.cap SC-CV	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F V C L Y M H V G R
L.CAP ACHK1	L M I Q I L T G L F L A M H Y T S D T A T A F S S V T H I C R D V N Y G W L I R Y L H A N G A S M F F V C L Y M H V G R

Individual	Sequence
L.sax SW-A	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax SW-B	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax C-D	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax C-E	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax N-T	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax N-Y	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax N-Zi	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.sax N-Z	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap N-Q	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap N-J1	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap N-J2	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap N-O	G I Y Y G S Y T Y L E T W N I G I V L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap N-N	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap SC-CL	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.cap SC-CV	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L
L.CAP ACHK1	G I Y Y G S Y T Y L E T W N I G I I L L F A V M A T A F M G Y V L P W G Q M S F W G A T V I T N L L S A I P Y I G T T L

Individual	Sequence
L.sax SW-A	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax SW-B	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax C-D	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax C-E	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax N-T	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax N-Y	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax N-Zi	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.sax N-Z	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap N-Q	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap N-J1	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap N-J2	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap N-O	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap N-N	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap SC-CL	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.cap SC-CV	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F
L.CAP ACHK1	V E W I W G G F S V D K A T L T R F F A F H F I L P F I I A A L V M I H L L F L H E T G S N N P S G I P S D S D K I P F

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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L.sax SW-A    H P Y Y T I K D V L G F L M L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax SW-B    H P Y Y T I K D V L G F L M L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax C-D     H P Y Y T I K D V L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax C-E     H P Y Y T I K D V L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax N-T     H P Y Y T I K D I L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax N-Y     H P Y Y T I K D I L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax N-Zi    H P Y Y T I K D V L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.sax N-Z     H P Y Y T I K D V L G F L V L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap N-Q     H P Y Y T I K D V L G F L I L I L L L T L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap N-J1    H P Y Y T I K D V L G F L I L I L L L T L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap N-J2    H P Y Y T I K D V L G F L I L I L L L T L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap N-O     H P Y Y T I K D V L G F L I V I F L L T L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap N-N     H P Y Y T I K D L L G F L I L I L L L T L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap SC-CL   H P Y Y T I K D V L G F L I L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.cap SC-CV   H P Y Y T I K D V L G F L I L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I
L.CAP ACHK1   H P Y Y T I K D V L G F L I L I L L L M L L V L F S P D L L G D P D N Y T P A N P L N T P P H I K P E W Y F L F A Y A I

L.sax SW-A    L R S I P N K L G G V M A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W A L V A D L L T L T W I G G
L.sax SW-B    L R S I P N K L G G V M A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W A L V A D L L T L T W I G G
L.sax C-D     L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.sax C-E     L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.sax N-T     L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.sax N-Y     L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.sax N-Zi    L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.sax N-Z     L R S I P N K L G G V L A L V M S I L I L A V I P F L H M S K Q R S M M F R P I S Q V L F W I L V A D L L T L T W I G G
L.cap N-Q     L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W I L V A D L L T L T W I G G
L.cap N-J1    L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W I L V A D L L T L T W I G G
L.cap N-J2    L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M T F R P I S Q V L F W V L V A D L L T L T W I G G
L.cap N-O     L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.cap N-N     L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.cap SC-CL   L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.cap SC-CV   L R S I P N K L G G V L A L V M S I L I L A I I P L L H M S K Q R S M M F R P I S Q V L F W V L V A D L L T L T W I G G
L.CAP ACHK1   L R S I P N K L G G V L A L V M S I L I L A I F P L L H M S K Q R S M M F R P I S Q V L F W I L V A D L L T L T W I G G

L.sax SW-A    Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax SW-B    Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax C-D     Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax C-E     Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax N-T     Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax N-Y     Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax N-Zi    Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.sax N-Z     Q P V E H P F I T I G Q V A S I L Y F S I I L I L M P L A S L I E N K I L K W *
L.cap N-Q     Q P V E H P F I T I G Q V A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap N-J1    Q P V E H P F I T I G Q V A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap N-J2    Q P V E H P F I T I G Q V A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap N-O     Q P V E H P F I T I G Q V A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap N-N     Q P V E H P F I T I G Q V A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap SC-CL   Q P V E H P F I T I G Q M A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.cap SC-CV   Q P V E H P F I T I G Q M A S I L Y F S I I L I F M P L A S L I E N K I L K W *
L.CAP ACHK1   Q P V E H P F I T I G Q M A S I L Y F S I I L I F M P L A S L I E N K I L K W *
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Table A8 Pairwise sequence divergences of the entire cytochrome *b* gene among South African scrub and Cape hares and Eurasian outgroup species. Coding as in Table A1; Lman = *Lepus mandshuricus*; Lcor = *L. corsicanus*; Ltim = *L. timidus*; Lgra = *L. granatensis*.

	A	B	D	E	T	Y	Z	Zim	CL	J	CK	CV	X	Q	O	N	Lman	Lcor	Ltim	Lgra
A		1	16	16	17	18	17	16	43	44	44	43	49	44	46	42	52	50	46	46
B	0.20%		16	16	16	17	16	16	42	43	43	42	48	43	45	42	53	50	45	45
D	4.40%	4.12%		1	3	4	3	3	42	40	39	42	44	40	42	39	52	49	44	45
E	4.40%	4.12%	0.20%		3	4	3	3	41	40	39	42	43	40	42	39	52	48	43	45
T	4.52%	4.24%	0.70%	0.70%		1	3	1	41	39	39	42	43	39	41	38	52	48	43	45
Y	4.81%	4.52%	0.91%	0.91%	0.20%		4	2	41	39	39	42	43	39	41	38	52	48	43	45
Z	4.54%	4.26%	0.71%	0.71%	0.81%	1.02%		3	42	41	40	43	45	41	42	40	53	50	45	45
Zim	4.38%	4.11%	0.81%	0.81%	0.30%	0.50%	0.70%		40	38	38	41	42	38	40	37	52	49	44	45
CL	15.93%	15.35%	15.29%	14.77%	14.91%	14.92%	15.86%	14.37%		20	20	7	20	21	21	22	52	48	47	52
J	16.85%	16.23%	14.27%	14.28%	13.90%	13.90%	14.81%	13.39%	5.76%		3	19	19	4	4	4	54	47	46	49
CK	16.53%	15.93%	14.00%	14.01%	13.64%	13.64%	14.53%	13.13%	5.60%	0.71%		20	19	5	6	5	52	47	46	48
CV	16.17%	15.58%	15.80%	15.26%	15.41%	15.41%	16.38%	14.85%	1.68%	5.13%	5.58%		20	20	20	20	49	47	44	51
X	20.20%	19.48%	16.60%	16.04%	16.20%	16.21%	17.21%	15.63%	5.52%	5.22%	5.07%	5.49%		20	22	21	55	51	49	51
Q	16.79%	16.18%	14.23%	14.25%	13.86%	13.87%	14.77%	13.35%	6.06%	1.03%	1.13%	5.73%	5.51%		7	7	54	49	48	50
O	17.90%	17.25%	15.20%	15.22%	14.82%	14.82%	15.77%	14.28%	6.03%	1.02%	1.57%	5.70%	6.10%	1.68%		8	55	49	48	52
N	15.62%	15.04%	13.69%	13.71%	13.09%	13.09%	14.21%	12.84%	6.36%	1.02%	1.13%	5.71%	5.80%	1.68%	1.90%		52	48	47	49
Lman	22.40%	23.13%	22.48%	22.50%	22.00%	21.99%	22.89%	22.34%	22.66%	23.98%	22.72%	20.04%	24.95%	23.86%	25.41%	22.61%		52	50	47
Lcor	22.10%	22.05%	20.67%	19.99%	19.96%	19.97%	21.82%	20.65%	20.29%	18.96%	19.26%	19.18%	21.70%	20.99%	20.84%	19.79%	23.03%		14	44
Ltim	18.64%	17.96%	17.14%	16.57%	16.54%	16.54%	18.10%	17.12%	19.02%	18.74%	18.38%	17.04%	20.35%	20.03%	19.89%	19.55%	20.89%	3.75%		43
Lgra	18.28%	17.62%	17.66%	17.08%	17.34%	17.34%	17.68%	17.02%	22.54%	20.02%	19.65%	21.32%	21.25%	20.65%	21.98%	19.91%	17.96%	16.76%	15.97%	

Table A9 Compound microsatellite defined genotypes of all specimens included in this study. The numbers correspond to the called allele size as retrieved *via* the GENOTYPER software. Zeros indicate that no allele was scored for that particular animal at the specific locus. Coding of individuals as in Table A1.

Species	Locality	Individual	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
L.saxatilis	Porterville	A1	166 - 170	249	0	107	170	188 - 190
		A2	166 - 168	247 - 249	202	0	170	186 - 188
		A3	168 - 170	247 - 251	200 - 202	107 - 109	170	190
		A4	168	249	200 - 202	107	170	190
		A5	168	247 - 249	200 - 202	107	170	190
		A6	166 - 168	249	200 - 204	107	170	186 - 188
		A7	166 - 168	249	0	107	170 - 172	
	Swellendam	B1	166 - 168	249 - 253	200	107 - 111	170	188 - 190
		B2	166 - 170	249 - 251		107	170	188 - 190
		B3	166 - 168	249 - 251	200	107	170	188
		B4	170 - 172	251 - 253	202 - 206	107	170	188 - 190
		B5	166 - 170	249 - 251	206	107	170	186 - 188
		B6	166 - 168	245 - 251	200 - 202	107	168 - 170	188
		B7	166 - 172	249 - 251	200 - 204	107	170	186 - 188
		B8	170	249 - 253	202 - 204	107 - 109	170	186 - 188
		B9	164 - 166	243 - 247	200 - 204	107	170	186 - 188
		B10	166 - 172	245 - 249	200 - 204	107 - 109	170 - 168	188 - 190
	Calvinia	C1	168 - 170	249	200 - 202	107	170	188
		C2	170 - 172	249	202	107	170	190
		C3	164 - 166	249	204	107	170	188 - 190
		C4	170	245 - 249	204	107	170	186 - 190
		C5	164 - 168	243 - 249	202 - 204	107	168 - 170	184 - 186
		C6	166 - 170	247 - 249	200	107	170	190
		C7	166 - 170	249	200 - 204	107	170	188
		C8	168 - 170	0	200	107	170	190
		C9	170	249	202 - 204	107	170	188 - 190
		C10	170	249	200	107	170	
	Oudtshoorn	D1	166 - 170	247 - 251	200	107	170	188 - 190
		D2	168 - 170	249	200	107	170	188
		D3	170 - 172	247 - 251	210	107	0	188
		D4	168 - 170	249 - 251	200 - 204	107	168 - 170	186 - 188
		D5	0	247 - 249	200	107	170 - 172	186 - 188
		D6	166 - 170	249	202 - 204	107	170	186 - 188
		D7	166 - 170	249	204	107	170	186 - 188
		D8	166 - 168	249	204	107	170	186 - 188
		D9	166 - 170	249	204	107	170	186 - 188
		D10	166 - 170	247 - 249	200 - 204	107	168 - 170	186 - 188
	Loxton	E1	164 - 170	249	200 - 204	107	168 - 170	186
		E2	168 - 170	249 - 251	206	107	170	186 - 190
		E3	168	249	206	107	168	186
		E4	168 - 170	249	204 - 206	107	170	186
		E5	166 - 168	249	200	107	170	188
	Grahamstown	F1	166 - 170	249	200	111	168 - 170	188
		F2	166	249	200	107 - 109	170	188 - 190
		F3	0	249	200 - 204	107	170	188
		F4	166	0	200	107 - 111	170	186
		F5	166 - 170	249	200	107	170 - 178	188 - 194
		F6	164 - 166	249	202 - 204	107	0	0
		F7	166 - 170	249	200 - 204	107	170	186 - 180
		F8	164 - 168	249 - 253	200 - 204	107	170	184 - 190
		F9	166 - 168	249	200 - 204	107	168 - 170	186 - 188

Species	Locality	Individual	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
		F10	164 - 170	249	204	107	170	188 - 190
		F11	166	249	202 - 204	107	170 - 172	186 - 188
	T-d-Riv	G1	166	249	198 - 200	107 - 111	168 - 170	186
		G2	166 - 168	243	198 - 200	107	170	186 - 188
		G3	166	0	200	107	170	186 - 188
		G4	166 - 168	249 - 251	200 - 202	107 - 111	170	188
		G5	168 - 170	249 - 255	202	107 - 109	170	186 - 188
		G6	166 - 168	247 - 249	200 - 202	107 - 109	170	186 - 188
		G7	166 - 170	249	200 - 204	107 - 109	170	188
		G8	166 - 170	249	200	107	170	188
		G9	166	249	204	107	170 - 172	186 - 188
		G10	166	249	204	107	0	186 - 188
	Port Shep	H1	166	241 - 247	0	107 - 109	166	188
		H2	166 - 170	249	200 - 204	107	170	184 - 188
		H3	0	245 - 249	0	0	170	186
	Kimberley	J6	166	247 - 249	200	0	170	188
		J7	166	241 - 247	198 - 200	109	168 - 170	186 - 188
		J10	166 - 170	249 - 251	202	107 - 109	170	192 - 194
	Will-Pret	K1	166	249	212	107 - 109	170 - 172	188 - 190
		K2	166 - 168	245 - 249	206	109	170	186 - 188
		K3	166 - 170	243 - 249	198 - 200	0	170	
		K4	166 - 168	245 - 251	200 - 202	107	170	184 - 186
		K5	166 - 172	245 - 247	200 - 210	107	170	188 - 190
		K6	166 - 170	249	200 - 204	107	170	188
		K7	0	0	200 - 204	107	170	0
		K8	166	245 - 249	200 - 204	107	170	188 - 190
		K9	166	249	200 - 204	107	170	184 - 188
		K10	166 - 168		200	107	170	186 - 188
	Vryheid	L1	166 - 168	241 - 247	200 - 204	107	170	188
		L2	162 - 166	243 - 249		107	170	186 - 188
		L3	168 - 170	249	200 - 204	107	170	186 - 188
		L4	166	247 - 249	200	107	170	186 - 188
		L5	166 - 170	249	200 - 204	107		188 - 190
		L6	166 - 170	249	204	107	170 - 172	186 - 188
		L7	166	243 - 247	198 - 204	107	170	188 - 190
		L8	166 - 168	245 - 249	202	107 - 109	170	186 - 190
		L9	166 - 170	249	204	107	170	188
	Ermelo	P4	168	0	202 - 204	107	170	0
		P13	166	241 - 245	202	107	170	188 - 190
	Vryburg	S1	166 - 168	245 - 251	200		170	186 - 188
	Rustenburg	T1	166	249	200 - 204	107 - 111	170	186 - 188
		T2	166 - 170	243 - 249	198 - 200	109	170	186 - 188
		T3	162 - 166	243 - 249	200	107 - 109	0	184 - 188
		T4	164	249	204	107	170	186 - 188
		T5	166 - 168	249	200 - 204	107	170	186 - 188
		T6	166 - 168	249	204	107	170	186 - 188
		T7	166 - 174	243 - 247	200	107 - 109	170 - 174	188
		T8	166	247 - 249	202	107	0	188
		T9	164 - 170	243 - 249	200	107 - 109	170 - 172	186 - 188
		T10	166 - 168	249	0	107	170 - 174	188
		T11	164 - 166	243 - 249	200	107 - 109	170 - 174	186 - 190
		T12	166 - 170	245 - 247	200	107 - 109	170 - 174	186 - 190
		T13	164 - 166	247 - 249	0	107	170 - 174	188
	Pretoria	U1	0	0	0	0	0	0
		U2	0	0	0	0	0	0
		U3	0	0	0	0	0	00
		U4	162 - 166	235 - 241	200	0	170	186

Species	Locality	Individual	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
		U5	166 - 168	249	200 - 202	107	170 - 174	186 - 188
		U6	166 - 168	245 - 249	200 - 204	107	170	188 - 190
		U7	166	249	202 - 204	107	170	188
		U8	164 - 166	245 - 249		107	170	188 - 190
		U9	168 - 170	241 - 247	200 - 202	107	170	185 - 188
		U10	162 - 166	245 - 249	200 - 204	107 - 109	170	186 - 188
		U11	166 - 168	0	200 - 202	107	170 - 174	188
		U12	164 - 170	243 - 247	200	107	170 - 174	186 - 190
		U13	164 - 168	243 - 247	202	107 - 111	170 - 174	188 - 190
		U14	164 - 166	243 - 249	200	107	170	184 - 186
		U15	168	249	200 - 206	109	170	184 - 190
		U16	164 - 166	249	200 - 202	113	170	186 - 190
		U17	166 - 170	245 - 247	0	107	170	188
	Vaalwater	V1	166 - 170	0	0	107	170	186 - 188
		V2	166 - 168	247 - 251	200 - 204	107	170	188 - 190
		V3	164 - 166	245 - 249	200	107 - 111	170	188 - 190
		V4	164 - 168	243 - 249	206	107	170	188 - 190
		V5	166 - 168	245 - 249	204	107	170	188 - 192
		V6	166 - 170	243 - 249	200 - 206	107	170	186 - 192
	Hoedspruit	W1	164 - 166	249	200	107	170 - 172	186 - 192
		W2	166	247 - 249	0	107	0	186 - 188
		W3	162 - 166	247	200 - 204	107	170	188
		W4	166 - 170	249	200	107 - 109	170	186
		W5	166	241 - 247	0	107	0	184 - 188
		W6	166 - 168	249	0	107	170 - 172	186 - 188
	Messina	Y1	164 - 166	249	200	107	170	188 - 190
		Y2	166	249	202 - 204	107	170	192 - 194
		Y3	162 - 166	249 - 251	200	107	170 - 172	186 - 190
		Y4	162 - 166	249	200 - 206	109	168	188
		Y5	164 - 170	241 - 251	200	107	170	186 - 188
		Y6	164 - 166	245 - 249	204	107	168 - 170	188
		Y7	166	249	0	107	170	188
		Y8	166 - 168	245 - 249	200 - 204	107	170 - 172	186 - 188
		Y9	166	245	0	107	0	190 - 192
		Y10	166 - 170	249	200	0	170	188
	Namibia	Z1	166 - 168	247 - 249	200 - 202	107	170	186
		Z2	164 - 166	247 - 251	202	107 - 111	170	186 - 188
		Z3	166 - 168	249 - 255	202	107	170	188
		Z4	166	249 - 253	200	107	0	186 - 188
		Z7	166 - 170	249	200 - 204	107	170	186 - 188
	Botswana	Ghanzi	166	249 - 255	200	107 - 111	170	188
	Zimbabwe	Zi1	166	241 - 249	200 - 204	107 - 109	170	188
		Zi2	164 - 166	247 - 249	0	107 - 109	170	186 - 190
		Zi3	166 - 168	249	0	107	170	188 - 192
		Zi4	162 - 166	241 - 249	200 - 204	107	170	186 - 190
		Zi5	166	249	204	107	170	186 - 188
		Zi6	166 - 170	249	200 - 204	107	170	186 - 190
		Zi7	170	249	204	107	170	188 - 190
		Zi8	164 - 166	249 - 251	0	107 - 111	170	188 - 190
		Zi9	166 - 170	247 - 249	202 - 206	107	170	188 - 190
		Zi10	162 - 166	247 - 249	202 - 204	107	170	188
L. capensis	Upington	I1	166 - 170	0	219	107 - 109	170 - 172	188
		Paulupi	168 - 170	0	226		172	180 - 186
	Kimberley	J1	168 - 172	0	207 - 210	107 - 111	170 - 172	188 - 190
		J2	166	0	208	107 - 109	172	186 - 188
		J3	0	0	0	0	0	0

Species	Locality	Individual	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
		J4	166- 170	0	204	107 - 109	170 - 172	188 - 190
		J5	166 - 168	0	204 - 212	107 - 111	170 - 172	190 - 196
		J8	166 - 168	249	200 - 204	107	170 - 172	188 - 196
		J9	166 - 168	249	204	107	170 - 172	184 - 188
	Hoopstad	M1	166	0	204	107	170 - 172	186 - 188
		M2	166 - 168	0	210	107 - 109	170 - 172	188 - 190
		M3	166 - 168	0	204	107	170	186 - 188
	Potchef	N1	166 - 170	0	210 - 212	107	170	186 - 188
	Parys	O1	166 - 172	0	0	107 - 113	172	188 - 194
		O2	166 - 170	0	204	107	170 - 172	188
		O3	168	249	0	107	172 - 176	186 - 188
		O4	166	0	204	107 - 113	0	188 - 192
		O5	168 - 170	249	210	111	172	186 - 192
		O6	166 - 170	247 - 249	0	107 - 109	172	192
		O7	166 - 168	249	200 - 214	107 - 111	170	188
		O8	166 - 170	249	0	107 - 109	172 - 174	182 - 186
		O9	166 - 172	249	0	109	172	188
		O10	166 - 170	249	0	107	170 - 172	186 - 188
	Ermelo	P1	166 - 170	0	204	107 - 109	172	190 - 192
		P2	170 - 172	249	0	107 - 113	172	182 - 190
		P3	166 - 168	0	0	0	170 - 172	186
		P5	168 - 170	249	204	107	172	188 - 190
		P6	166 - 168	249	204	107	172	190 - 194
		P7	168 - 170	249	204	107	172	186 - 188
		P8	164	249	204 - 212	107 - 109	174	184 - 188
		P9	170	249	200 - 210	109	172	188 - 190
		P10	170	249	0	109	172	188 - 196
		P11	166	249	0	111 - 115	172 - 176	188 - 190
		P12	166 - 170	249	200	111 - 115	172	190
	Kalahari	Q1	166 - 168	0	204	107	172	186 - 190
		Q2	166 - 168	0	204	107 - 109	172 - 174	188 - 192
		Q3	166 - 168	0	204	0	170 - 174	188 - 194
		Q4	174	247 - 249	204 - 216	107	172	188 - 190
		Q5	166	249	200	107	172	0
		Q6	168	249	200 - 204	107 - 109	172 - 174	0
		Q7	188	249	200	107 - 111	172	188 - 192
		Q8	168 - 170	249	204 - 206	107 - 109	172 - 174	188
		Q9	166	249		107	170 - 172	0
		Q10	170 - 174	249	204	107	0	188
		Q*	166 - 168	0		107 - 113	170 - 172	186 - 188
		Paul1	166 - 172	0	204	107 - 109	172	186 - 190
		Paul2	170 - 174	0	204 - 212	0	172 - 174	188 - 190
		Paul3	0	0	0	0	0	0
	Hotazel	R1	166 - 170	0	204	109	172	188
	KNP	X1	166 - 170	249	204	107	170	192 - 194
	Namibia	Z5	162	0	0	101	170 - 172	182 - 184
		Z6	166 - 170	0	0	107	170 - 172	186 - 188
		Z8	168 - 172	0	204 - 206	107 - 111	172 - 174	188 - 190
		Z9	166 - 170	0	212 - 224		170	186 - 188
	Kimberley	CK1	168 - 172	245 - 249	204 - 210	107 - 109	172 - 176	182 - 188
		CK2	170 - 172	249	204	107	172 - 176	188
	VictoriaW	CV1	166	249	204	107	172 - 174	188 - 190
		CV2	166 - 170	249	204	107	172	188
		CV3	166 - 170	249	204	107	172 - 176	184 - 186
		CV4	168 - 172	249	202 - 206	107 - 111	172 - 174	186 - 188
		CV5	0	0	200 - 204	107	170 - 172	186 - 188
		CV6	168 - 172	249	200	107	172 - 174	0
		CV7	164	249	200 - 204	107	170	186

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Species	Locality	Individual	Lsa1	Lsa2	Lsa3	Lsa4	Lsa6	Lsa8
		CV8	0	245 - 249	200	107	172 - 176	186 - 190
		CV9	0	249	200	107	172 - 176	188 - 190
		CV10	166	249	200 - 204	107	170 - 172	0
	Loxton	CL1	168	249	208	107 - 109	172	188
		CL2	170 - 172	245	204	107	172	188 - 190
		CL3	166 - 168	249	0	107	170 - 172	186 - 188
Bunolagus		Bu1	166 - 170	0	200 - 204	107	170 - 172	184 - 188
		Bu2	166	249	204	107	170 - 172	184 - 186
Pronolagus		PParys1	166 - 170	249	200 - 204	107	170 - 172	178 - 188
		PParys2	166	249	200	107	170 - 172	186 - 188
P. rupestris		Prup1	0	0	0	107	170 - 171	0
		Prup2	166	249	200 - 204	107	170 - 172	186 - 188
		Prup3	0	0	0	107	164 - 165	182
P.randensis		Pran1	170	0	0	107	164 - 170	178
		Pran2	0	0	0	0	164 - 172	182
P.crassicau		Pcrass1	170 - 172	249	200 - 204	107	170 - 172	182
		Pcrass2	166 - 168	249	200	107	170 - 172	178 - 188
Oryctolagus		Orycto	166 - 168	0	0	107	170 - 171	186 - 188
Caprolagus		Caprolagus	166	249	0	107	170	186 - 188
Ochtonoid		Ochtonoid	166 - 168	0	0	107	170 - 172	186 - 188

Table A10 The observed (H_O) and expected heterozygosities (H_E) and likelihood ratio test probabilities of Hardy-Weinberg proportions listed for each locus and each population of southern African hares, values that are significant after Bonferroni corrections are in bold; L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Locus		L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
Lsa1						
	H_E	0.75	0.64	0.67	0.75	0.80
	H_O	0.77	0.70	0.77	0.75	0.60
	P(HW)	0.22	0.60	0.68	**0.02**	0.10
Lsa2						
	H_E	0.61	0.51	0.66	0.10	0.23
	H_O	0.64	0.41	0.67	0.11	0.08
	P(HW)	0.96	0.30	0.84	0.99	0.05
Lsa3						
	H_E	0.70	0.69	0.67	0.66	0.67
	H_O	0.58	0.45	0.50	0.35	0.33
	P(HW)	0.21	**0.0004**	0.06	0.19	0.05
Lsa4						
	H_E	0.15	0.24	0.33	0.56	0.15
	H_O	0.16	0.19	0.23	0.50	0.15
	P(HW)	0.97	0.16	**0.002**	0.04	0.99
Lsa6						
	H_E	0.15	0.30	0.22	0.54	0.63
	H_O	0.13	0.26	0.21	0.50	0.70
	P(HW)	0.97	0.17	0.18	0.39	0.43
Lsa8						
	H_E	0.66	0.62	0.66	0.75	0.69
	H_O	0.58	0.65	0.74	0.78	0.73
	P(HW)	0.11	0.73	0.54	0.99	0.75
mean						
	H_E	0.50	0.50	0.53	0.56	0.53
	H_O	0.48	0.44	0.52	0.50	0.43

Table A11 Results of probabilities of genotypic linkage disequilibrium for pairwise comparisons among six microsatellite loci in southern African hare populations calculated using the Markov chain algorithm implemented in GENEPOP; L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage. The value that is significant at the 0.05 level after Bonferroni correction is in bold.

Population	Locus Pair	*P*-value
L. sax. SW	Lsa1 & Lsa2	0.873
L. sax. SW	Lsa1 & Lsa3	0.153
L. sax. SW	Lsa1 & Lsa4	0.752
L. sax. SW	Lsa1 & Lsa6	0.350
L. sax. SW	Lsa1 & Lsa8	0.643
L. sax. SW	Lsa2 & Lsa3	0.981
L. sax. SW	Lsa2 & Lsa4	0.020
L. sax. SW	Lsa2 & Lsa6	0.113
L. sax. SW	Lsa2 & Lsa8	0.559
L. sax. SW	Lsa3 & Lsa4	0.963
L. sax. SW	Lsa3 & Lsa6	0.942
L. sax. SW	Lsa3 & Lsa8	0.143
L. sax. SW	Lsa4 & Lsa6	0.510
L. sax. SW	Lsa4 & Lsa8	1.000
L. sax. SW	Lsa6 & Lsa8	0.142
L. sax. C	Lsa1 & Lsa2	0.045
L. sax. C	Lsa1 & Lsa3	0.109
L. sax. C	Lsa1 & Lsa4	0.993
L. sax. C	Lsa1 & Lsa6	0.695
L. sax. C	Lsa1 & Lsa8	0.411
L. sax. C	Lsa2 & Lsa3	**0.0002**
L. sax. C	Lsa2 & Lsa4	0.382
L. sax. C	Lsa2 & Lsa6	0.958
L. sax. C	Lsa2 & Lsa8	0.234
L. sax. C	Lsa3 & Lsa4	0.019
L. sax. C	Lsa3 & Lsa6	0.316
L. sax. C	Lsa3 & Lsa8	0.493
L. sax. C	Lsa4 & Lsa6	0.112
L. sax. C	Lsa4 & Lsa8	0.461
L. sax. C	Lsa6 & Lsa8	0.278
L. sax. N	Lsa1 & Lsa2	0.042
L. sax. N	Lsa1 & Lsa3	0.027
L. sax. N	Lsa1 & Lsa4	0.130
L. sax. N	Lsa1 & Lsa6	0.350
L. sax. N	Lsa1 & Lsa8	0.182
L. sax. N	Lsa2 & Lsa3	0.070
L. sax. N	Lsa2 & Lsa4	0.644
L. sax. N	Lsa2 & Lsa6	0.416
L. sax. N	Lsa2 & Lsa8	0.380
L. sax. N	Lsa3 & Lsa4	0.021

L. sax. N	Lsa3 & Lsa6	0.306
L. sax. N	Lsa3 & Lsa8	0.738
L. sax. N	Lsa4 & Lsa6	0.131
L. sax. N	Lsa4 & Lsa8	0.828
L. sax. N	Lsa6 & Lsa8	0.834
L. cap. N	Lsa1 & Lsa2	0.124
L. cap. N	Lsa1 & Lsa3	0.197
L. cap. N	Lsa1 & Lsa4	0.204
L. cap. N	Lsa1 & Lsa6	0.089
L. cap. N	Lsa1 & Lsa8	0.880
L. cap. N	Lsa2 & Lsa3	0.119
L. cap. N	Lsa2 & Lsa4	0.710
L. cap. N	Lsa2 & Lsa6	0.693
L. cap. N	Lsa2 & Lsa8	0.213
L. cap. N	Lsa3 & Lsa4	0.035
L. cap. N	Lsa3 & Lsa6	0.318
L. cap. N	Lsa3 & Lsa8	0.779
L. cap. N	Lsa4 & Lsa6	0.355
L. cap. N	Lsa4 & Lsa8	0.248
L. cap. N	Lsa6 & Lsa8	0.897
L. cap. SC	Lsa1 & Lsa2	0.404
L. cap. SC	Lsa1 & Lsa3	0.516
L. cap. SC	Lsa1 & Lsa4	0.743
L. cap. SC	Lsa1 & Lsa6	0.403
L. cap. SC	Lsa1 & Lsa8	1.000
L. cap. SC	Lsa2 & Lsa3	1.000
L. cap. SC	Lsa2 & Lsa4	1.000
L. cap. SC	Lsa2 & Lsa6	1.000
L. cap. SC	Lsa2 & Lsa8	0.582
L. cap. SC	Lsa3 & Lsa4	0.013
L. cap. SC	Lsa3 & Lsa6	0.205
L. cap. SC	Lsa3 & Lsa8	0.702
L. cap. SC	Lsa4 & Lsa6	1.000
L. cap. SC	Lsa4 & Lsa8	0.837
L. cap. SC	Lsa6 & Lsa8	0.266

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Table A12 Results of probabilities of genotypic linkage disequilibrium for pairwise comparisons among six microsatellite loci in southern African scrub hares and Cape hares across all populations calculated using the Fisher's exact tests as implemented in GENEPOP. The value that is significant at the 0.05 level after Bonferroni correction is in bold.

Locus Pair	Chi-square	df	P-value
Lepus saxatilis			
Lsa1 & Lsa2	12.836	6	0.046
Lsa1 & Lsa3	15.426	6	0.017
Lsa1 & Lsa4	4.665	6	0.587
Lsa1 & Lsa6	4.931	6	0.553
Lsa1 & Lsa8	6.066	6	0.416
Lsa2 & Lsa3	22.287	6	**0.001**
Lsa2 & Lsa4	10.660	6	0.099
Lsa2 & Lsa6	6.196	6	0.402
Lsa2 & Lsa8	6.011	6	0.422
Lsa3 & Lsa4	15.738	6	0.015
Lsa3 & Lsa6	4.794	6	0.570
Lsa3 & Lsa8	5.907	6	0.434
Lsa4 & Lsa6	9.802	6	0.133
Lsa4 & Lsa8	1.925	6	0.926
Lsa6 & Lsa8	6.828	6	0.337
Lepus capensis			
Lsa1 & Lsa2	5.996	4	0.199
Lsa1 & Lsa3	4.575	4	0.334
Lsa1 & Lsa4	3.772	4	0.438
Lsa1 & Lsa6	6.651	4	0.156
Lsa1 & Lsa8	0.255	4	0.993
Lsa2 & Lsa3	4.254	4	0.373
Lsa2 & Lsa4	0.686	4	0.953
Lsa2 & Lsa6	0.733	4	0.947
Lsa2 & Lsa8	4.174	4	0.383
Lsa3 & Lsa4	15.357	4	0.004
Lsa3 & Lsa6	5.455	4	0.244
Lsa3 & Lsa8	1.208	4	0.877
Lsa4 & Lsa6	2.070	4	0.723
Lsa4 & Lsa8	3.145	4	0.534
Lsa6 & Lsa8	2.864	4	0.581

Table A13 Allele frequencies for locus Lsa1, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L.sax. N	L. cap. N	L. cap. SC
A		0.0179	0.0507	0.0189	
B	0.0577	0.0446	0.1304	0.0189	0.1000
C	0.2692	0.5268	0.5290	0.3774	0.3500
D	0.2692	0.1786	0.1522	0.2264	0.2500
E	0.3269	0.2232	0.1232	0.2453	0.1500
F	0.0769	0.0089	0.0072	0.0755	0.1500
G			0.0072	0.0377	

Table A14 Allele frequencies for locus Lsa2, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
A			0.0076		
B		0.0446	0.0379		
C	0.0400	0.0357	0.0833		
D	0.0600	0.0268	0.1288	0.0179	0.1250
E	0.1000	0.1339	0.1136	0.0357	
F	0.6000	0.6875	0.5530	0.9464	0.8750
G	0.1400	0.0536	0.0530		
H	0.0600	0.0089	0.0076		
I		0.0089	0.0152		

Table A15 Allele frequencies for locus Lsa3, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
A		0.0377	0.0167		
B	0.4167	0.4340	0.5000	0.1250	0.3750
C	0.2708	0.1321	0.1500		0.0417
D	0.2500	0.3302	0.2417	0.5625	0.4583
E	0.0625	0.0472	0.0667	0.0250	0.0417
F				0.0375	0.0833
G		0.0189	0.0083	0.1000	
H			0.0167	0.0625	
I				0.0125	
J				0.0125	
K				0.0250	
L				0.0125	
M				0.0250	

Table A16 Allele frequencies for locus Lsa4, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
A	0.9200	0.8684	0.8106	0.6250	0.9231
B	0.0600	0.0877	0.1288	0.2188	0.0385
C	0.0200	0.0439	0.0455	0.0938	0.0385
D			0.0152	0.0417	
E				0.0208	

Table A17 Allele frequencies for locus Lsa6, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
A	0.0577	0.0849	0.0227		
B	0.9231	0.8302	0.8788	0.2500	0.1923
C	0.0192	0.0566	0.0303	0.6250	0.5769
D			0.0682	0.0865	0.1154
E				0.0385	0.1154
F		0.0094			
G		0.0189			

Table A18 Allele frequencies for locus Lsa8, L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis*, SW = southwestern assemblage, SC = south-central assemblage, C = central assemblage, N = northern assemblage.

Allele	L. sax. SW	L. sax. C	L. sax. N	L. cap. N	L. cap. SC
A				0.0392	
B	0.0208	0.0263	0.0441	0.0294	0.0455
C	0.1667	0.3509	0.2426	0.1667	0.3182
D	0.4167	0.5000	0.5000	0.4412	0.4545
E	0.3958	0.0789	0.1691	0.1667	0.1818
F		0.0175	0.0368	0.0784	
G		0.0175	0.0074	0.0392	
H				0.0294	
I		0.0088		0.0098	

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Table A19 Pairwise nuclear genetic distances measured in Nei's D_A (Nei *et al.* 1983) among South African hares (L. sax. = *Lepus saxatilis*, L. cap. = *L. capensis;* SW = southwestern, SC = south-central, CN = central-northern, N = northern). Coding of sample localities as in Table A1.

	LsaxA	LsaxB	LsaxC	LsaxD	LsaxE	LsaxF	LsaxG	LsaxH	LsaxJ	LsaxK	LsaxL	Lsax12	LsaxS	LsaxT	LsaxU	LsaxV
LsaxA	0.000															
LsaxB	0.129	0.000														
LsaxC	0.090	0.104	0.000													
LsaxD	0.111	0.096	0.092	0.000												
LsaxE	0.191	0.139	0.154	0.129	0.000											
LsaxF	0.136	0.114	0.091	0.106	0.151	0.000										
LsaxG	0.123	0.109	0.146	0.086	0.181	0.090	0.000									
LsaxH	0.257	0.204	0.205	0.165	0.288	0.170	0.159	0.000								
LsaxJ	0.252	0.226	0.320	0.247	0.380	0.255	0.165	0.225	0.000							
LsaxK	0.123	0.085	0.117	0.112	0.191	0.130	0.101	0.136	0.228	0.000						
LsaxL	0.090	0.116	0.083	0.079	0.195	0.104	0.064	0.122	0.221	0.073	0.000					
Lsax12	0.250	0.289	0.279	0.359	0.446	0.357	0.353	0.379	0.442	0.278	0.239	0.000				
LsaxS	0.332	0.238	0.407	0.304	0.352	0.358	0.268	0.333	0.331	0.230	0.318	0.400	0.000			
LsaxT	0.147	0.136	0.128	0.146	0.229	0.104	0.081	0.149	0.221	0.099	0.070	0.363	0.314	0.000		
LsaxU	0.126	0.121	0.107	0.177	0.217	0.134	0.137	0.173	0.250	0.102	0.090	0.243	0.283	0.057	0.000	
LsaxV	0.172	0.090	0.124	0.148	0.138	0.152	0.157	0.218	0.323	0.104	0.117	0.305	0.255	0.136	0.117	0.000
LsaxW	0.177	0.199	0.193	0.124	0.228	0.127	0.119	0.116	0.207	0.137	0.098	0.422	0.305	0.101	0.142	0.174
LsaxY	0.161	0.098	0.133	0.127	0.158	0.090	0.127	0.165	0.195	0.103	0.103	0.294	0.263	0.122	0.123	0.111
LsaxZ	0.121	0.113	0.156	0.111	0.202	0.115	0.073	0.212	0.223	0.165	0.123	0.319	0.256	0.138	0.137	0.169
LsaxBot	0.349	0.292	0.346	0.304	0.412	0.227	0.200	0.298	0.333	0.276	0.290	0.480	0.317	0.272	0.306	0.291
LsaxZim	0.108	0.101	0.098	0.104	0.169	0.094	0.113	0.148	0.212	0.118	0.060	0.267	0.381	0.106	0.101	0.099
LcapI	0.387	0.403	0.464	0.365	0.448	0.377	0.335	0.410	0.426	0.364	0.349	0.517	0.485	0.371	0.434	0.460
LcapJ	0.248	0.250	0.236	0.207	0.316	0.200	0.229	0.274	0.446	0.164	0.199	0.392	0.487	0.234	0.279	0.272
LcapM	0.207	0.228	0.276	0.158	0.289	0.205	0.182	0.244	0.393	0.150	0.132	0.218	0.322	0.208	0.255	0.230
LcapN	0.358	0.293	0.317	0.204	0.329	0.307	0.270	0.289	0.373	0.222	0.260	0.400	0.375	0.329	0.341	0.310
LcapO	0.280	0.287	0.297	0.182	0.325	0.208	0.198	0.270	0.346	0.230	0.212	0.524	0.514	0.217	0.295	0.287
LcapP	0.274	0.309	0.259	0.248	0.324	0.196	0.272	0.321	0.443	0.243	0.247	0.541	0.605	0.224	0.294	0.313
LcapQ	0.215	0.252	0.246	0.194	0.270	0.186	0.206	0.273	0.375	0.195	0.179	0.461	0.516	0.166	0.244	0.240
LcapR	0.597	0.527	0.607	0.488	0.699	0.490	0.452	0.424	0.499	0.480	0.440	0.659	0.698	0.449	0.595	0.612
LcapX	0.379	0.378	0.287	0.288	0.359	0.271	0.333	0.323	0.416	0.350	0.285	0.500	0.700	0.378	0.420	0.305
LcapZ	0.333	0.257	0.303	0.262	0.296	0.276	0.297	0.354	0.525	0.223	0.246	0.378	0.476	0.273	0.272	0.258
LcapCV	0.194	0.185	0.151	0.156	0.197	0.117	0.180	0.236	0.417	0.153	0.154	0.402	0.424	0.138	0.173	0.190
LcapCL	0.277	0.292	0.278	0.251	0.328	0.279	0.285	0.331	0.510	0.233	0.224	0.377	0.506	0.289	0.330	0.312

	LsaxW	LsaxY	LsaxZ	LsaxBot	LsaxZim	LcapI	LcapJ	LcapM	LcapN	LcapO	LcapP	LcapQ	LcapR	LcapX	LcapZ	LcapCV	LcapCL
LsaxA																	
LsaxB																	
LsaxC																	
LsaxD																	
LsaxE																	
LsaxF																	
LsaxG																	
LsaxH																	
LsaxJ																	
LsaxK																	
LsaxL																	
Lsax12																	
LsaxS																	
LsaxT																	
LsaxU																	
LsaxV																	
LsaxW	0.000																
LsaxY	0.113	0.000															
LsaxZ	0.144	0.172	0.000														
LsaxBot	0.257	0.268	0.211	0.000													
LsaxZim	0.118	0.085	0.119	0.287	0.000												
LcapI	0.343	0.389	0.428	0.559	0.426	0.000											
LcapJ	0.253	0.227	0.329	0.396	0.239	0.344	0.000										
LcapM	0.203	0.208	0.252	0.403	0.181	0.334	0.143	0.000									
LcapN	0.296	0.328	0.256	0.376	0.289	0.417	0.365	0.275	0.000								
LcapO	0.191	0.234	0.293	0.386	0.236	0.316	0.126	0.171	0.358	0.000							
LcapP	0.255	0.239	0.379	0.479	0.252	0.354	0.104	0.232	0.457	0.104	0.000						
LcapQ	0.190	0.195	0.302	0.403	0.199	0.338	0.145	0.184	0.437	0.099	0.088	0.000					
LcapR	0.475	0.464	0.641	0.659	0.483	0.373	0.335	0.383	0.659	0.299	0.280	0.314	0.000				
LcapX	0.336	0.291	0.376	0.480	0.248	0.588	0.374	0.375	0.400	0.302	0.365	0.328	0.600	0.000			
LcapZ	0.286	0.287	0.333	0.443	0.242	0.408	0.206	0.278	0.316	0.261	0.248	0.220	0.514	0.444	0.000		
LcapCV	0.162	0.147	0.216	0.347	0.175	0.390	0.131	0.215	0.396	0.158	0.120	0.115	0.456	0.368	0.201	0.000	
LcapCL	0.307	0.259	0.383	0.524	0.301	0.289	0.088	0.168	0.447	0.205	0.197	0.172	0.316	0.430	0.271	0.185	0.000